THE HEPATO-RENAL SYNDROME

(an expanded concept)

by

Charles Neville Crowson, B.A., M.A., M.D., C.M., L.M.C.C.+

Thesis presented for the degree, Doctor of Philosophy, University of Edinburgh.

April, 1954.

+ Graduate Medical Research Fellow of the National Research Council of Canada.

ORIGINAL

CONTENTS

Section V (contd).

PREFACE

Preface.

The contents of this thesis may be likened in analogy to the multiplications of the germ of an idea implanted in the author's mind in the summer of 1951 while he served as prosector in the pathology department of Queen's University, Kingston, Ontario, Canada. Fortuitously, and in accord with the enigmatic "rule of threes" which appears operative in pathology departments the world over, three cases of renal tubular necrosis with associated hepatic lesions presented themselves in rapid succession. The renal lesion has been designated "glomerulotubular nephrosis" (G.T.N.) for reasons which will become obvious as the text unfolds. What is perhaps debatable is the choice of the term "hepato-renal syndrome" to designate the correlated lesions, though this too is dealt with in the text. Suffice it to say that a remarkably constant relationship was found to exist between G.T.N. and a variety of morphological alterations in the liver, and that to this correlation an expanded concept of the term has been applied.

Superficial spade-work having bared solid roots, the research project was transferred to Edinburgh University in the summer of 1952 where it prospered

for two years in association with Professor A.M. Drennan. At the outset it was apparent that certain decided limitations would be necessary in order that fruitful subtotal objectives could be reached within a reasonable period of time. Indeed, the ramifications of this study are, in themselves, so vast as to resemble the delicate arboreal pattern of the renal vasculature, which appears to play such an important pathogenetic role in glomerulotubular nephrosis. Much having been accomplished, many problems remain for future resolution.

The scope of these recorded investigations falls readily into five sections. Following a brief re-introduction to the anatomy of the kidney and liver, with attention focussed upon the vasculature of these organs, the text treats first of autolysis and its differentiation from true necrosis; next of human G.T.N. as noted in the files of the autopsy service of the Kingston General Hospital; next of experimental G.T.N. as induced surgically in the rabbit; next of experimental G.T.N. produced by toxic agents in the rat; and finally of microarteriographic studies into the pathogenesis of the lesions in the rat.

Though microarteriography was instituted early in the investigations as pursued in Edinburgh, initial, and, as subsequently disclosed, artefactual results suggesting spasm in the larger renal vessels placed the emphasis in the wrong direction. As techniques improved it became apparent that if spasm occurred at all, it did so at pre-capillary levels. This finding evolved at a relatively late date in the studies of the toxic nephroses and along with numerous earlier technical faults obviated the prior arteriographic studies. Due to the somewhat excessive demands placed by this procedure on my radiologic and photographic associates, it was decided to limit this aspect of the investigation to a demonstration of the vascular changes induced by acute carbon tetrachloride and acute corrosive sublimate poisoning. Admittedly this falls short of the ultimate objective, the unequivocal proof of the vasospastic pathogenesis of the renal lesions in the hepato-renal syndrome, but it is felt to be a decided advance in the knowledge and understanding of the pathogenesis of the mercurial and chlorinated hydrocarbon forms of nephrosis, whilst offering some insight into acute tubular necrosis irrespective of etiology.

Throughout the text, numerous references are made to important publications of two masters in their respective fields, H.P. Himsworth's "Diseases of the Liver" and A.C. Allen's "The Kidney, Medical and Surgical Diseases". These books are milestones of present-day developments in our knowledge of liver and kidney disorders and have played a major role in moulding the author's views.

The final chapter of this present work has been accomplished with the invaluable assistance and industrious co-operation of two experts in highly technical fields; Dr. J. B. King, Radiologist attached to the Department of Surgery, University of Edinburgh and Mr. T.C. Dodds, head of the Photomicrography Unit in the Pathology Department of the University of Edin-:burgh. Their contributions speak for themselves.

Acknowledgements

The author is indebted to Professor Sir James Learmonth for permission to use the radiographic facilities of the Department of Surgery, to Mr. P.H. Mott of Queen's University and Mr. T.C. Dodds for photographs and photomicrographs employed in this thesis, to Miss Margaret Melville (now deceased) of Queen's University and Mr. James Waugh of Edinburgh

University for histological sections of vast quantities of human and animal tissues, and last, but by no means least, to Professor A.M. Drennan of Edinburgh University and Professor Robert H. More of Queen's University for wise advice and friendly counsel throughout the prosecution of the research.

The research was aided, in part, by a grant from the Moray Fund and by the generosity of the Ciba Company who supplied a variety of drugs. The author was maintained financially through the auspices of the National Research Council of Canada.

INTRODUCTION

Introduction.

The vast majority of degenerative lesions to which the flesh falls heir are known to be related to local or general disturbances of hemodynamics, usually of an occlusive nature and most frequently organic in form. Such disturbances lead ultimately to ischemia and depending on the extent, degree and rapidity of development one finds diffuse or focal areas of tissue disintegration varying from atrophy to frank necrosis and often intermingled. In addition there exists a smaller and varied group in which atrophy and/or necrosis are shown to occur in the absence of organic vascular obstruction and are due, presumably, to functional vasospasm. Two examples which may be cited are the digits in Raynaud's Disease and the renal convoluted tubules in "acute tubular necrosis". Since this text deals primarily with the latter phenomenon and its association with predominantly degenerative hepatic lesions it is considered reasonable to commence with a brief discussion of the vascular anatomy of the liver and kidney.

The striking dissimilarity between the human and rodent livers is remarkably superficial. The melting-pot of the evolutionary process has tended to fuse the six-lobed rodent liver down to the predominantly bi-lobed human liver but this appearance is deceptive and one

may readily demonstrate the vestigial remains of bygone subsidiary hepatic lobes in man. At the histo-:logical level the human liver may be differentiated from the rodent only by way of the greater size of the lobules and their parenchymal cell and vascular components.

The liver is an organ with a double circulation. Broadly speaking, the hepatic artery supplies its oxygen requirements and the portal vein its nutritional necessities, though as Himsworth (1950) remarks, there would appear to be some slight species specificity in regard to the relative proportions of these essentials carried by these pathways. In the human the hepatic artery entering by way of the porta hepatis bifurcates into main right and left branches within the substance of the liver. In the rodent the branches to the various lobes commence at varying distances from the liver in the porta hepatis. The portal vein in both species forms by the junction of the splenic and the mesenteric veins and approaches the hilum of the liver via the porta hepatis. Its subsequent dissociation is patterned after that of the respective hepatic artery. The portal vein and hepatic artery, with their accompanying invagination of Glisson's Capsule then proceed to ramify throughout the organ. They lie in close proximity to the bile ducts in the regions known as portal tracts which constitute the periphery of the liver lobule. The lobule forms the

structural unit of the liver and has at its core the central vein. Blood enters at the periphery of the lobule from both arterial and venous sources, percolates along the sinusoids between the hepatic cords and exits via the central vein into the hepatic vein.

Fig.1. Rat Liver Lobe. The arterial and portal vas-:cular patterns as outlined with Bismuth contrast medium. Radioarteriograph, enlarged.

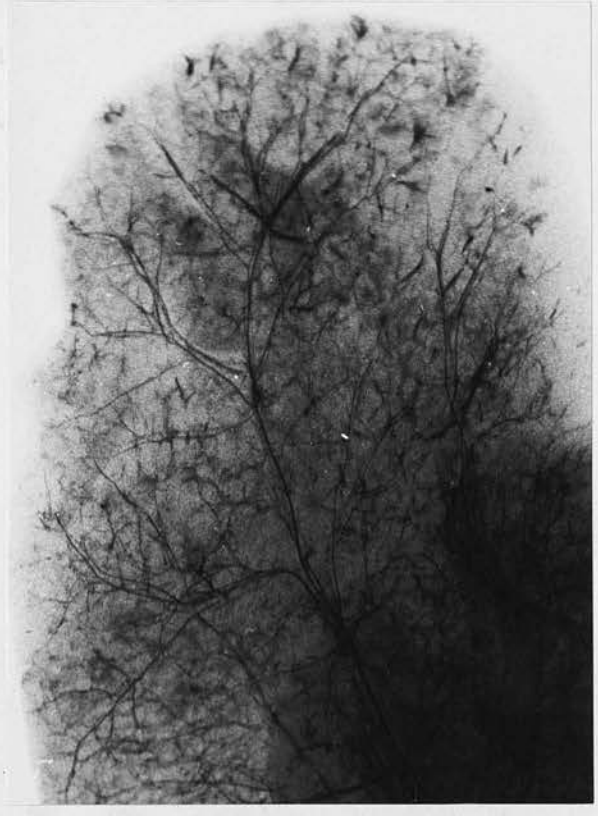


Fig.2. Rat Liver Lobe. The vascular pattern of the hepatic artery as seen following occlusion of the portal vein. Radioarteriograph, enlarged.

Figures 1 and 2 demonstrate the relative sizes of the hepatic artery and the portal vein within the rat liver, as outlined by means of radioarteriography using bismuth oxychloride for contrast. In figure 2 the portal vein has been clamped off and the filling represents that due solely to the hepatic artery. The finer details of lobular

injection can be better discerned from figures 3 and 4, as shown by means of photoarteriography from 50 micron sections of the respective livers. These illustrations give some idea of the relative blood volumes supplied to the liver by these two routes and form a nice demonstration of the importance of the vascular design in the overall structure of the liver.

Fig.3. Rat Liver.
50 micron unstained section of the liver shown in fig.1. X30. Photoarteriograph, showing the finer vascular channels as filled by both venous and arterial routes.

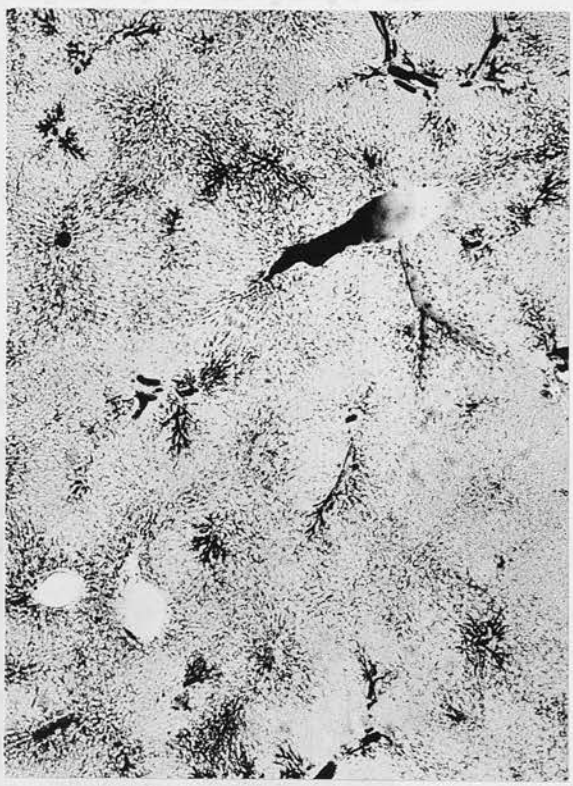
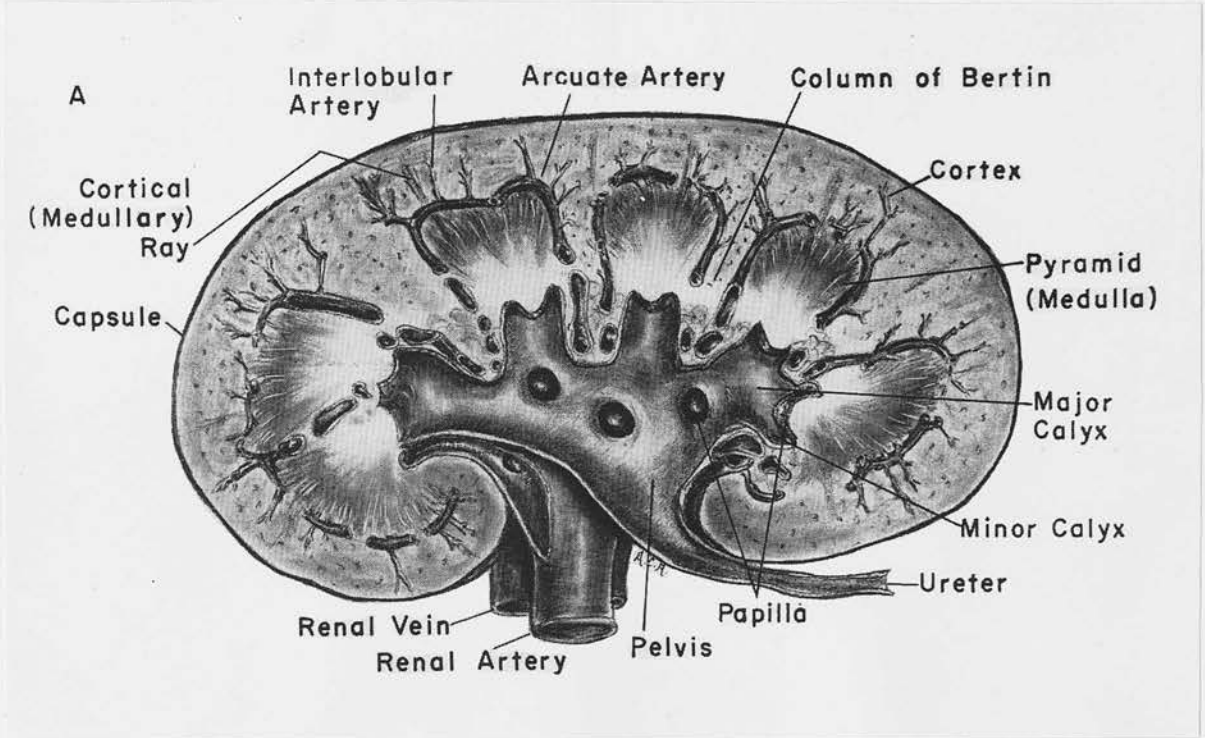


Fig. 4. Rat Liver.
50 micron unstained section of the liver shown in Fig.2. X30. Photoarteriograph, showing sinusoidal pattern as filled by the hepatic artery alone.

The chief anatomical difference between the human and the rodent kidney is that in the former we deal with a multi-pyramidal structure and in the latter with a uni-pyramidal one. The human kidney might be considered to represent the fusion product of half a dozen or so nephric units, each structurally similar to a rodent kidney. The major gross features of the hemisectioned human kidney are illustrated in figure 5 A. Figure 5 B displays the radioarteriogram of an entire rat kidney, expanded to a comparable size.

A



B

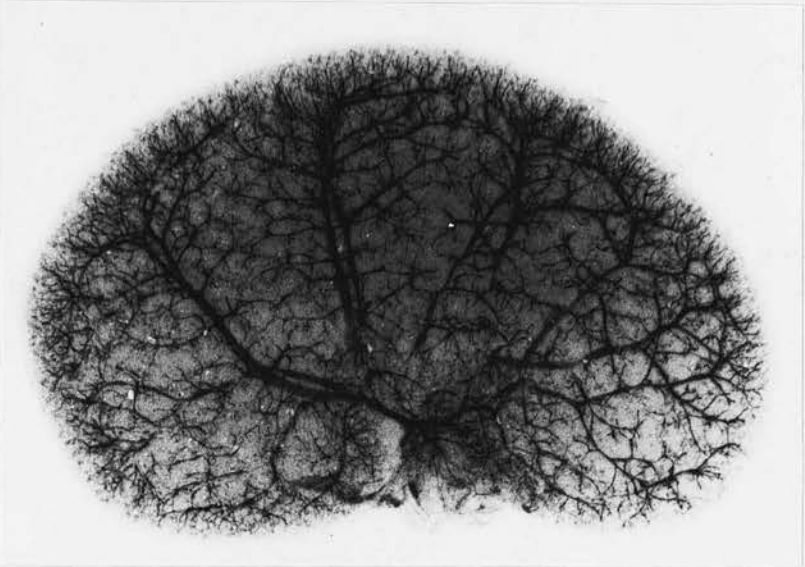


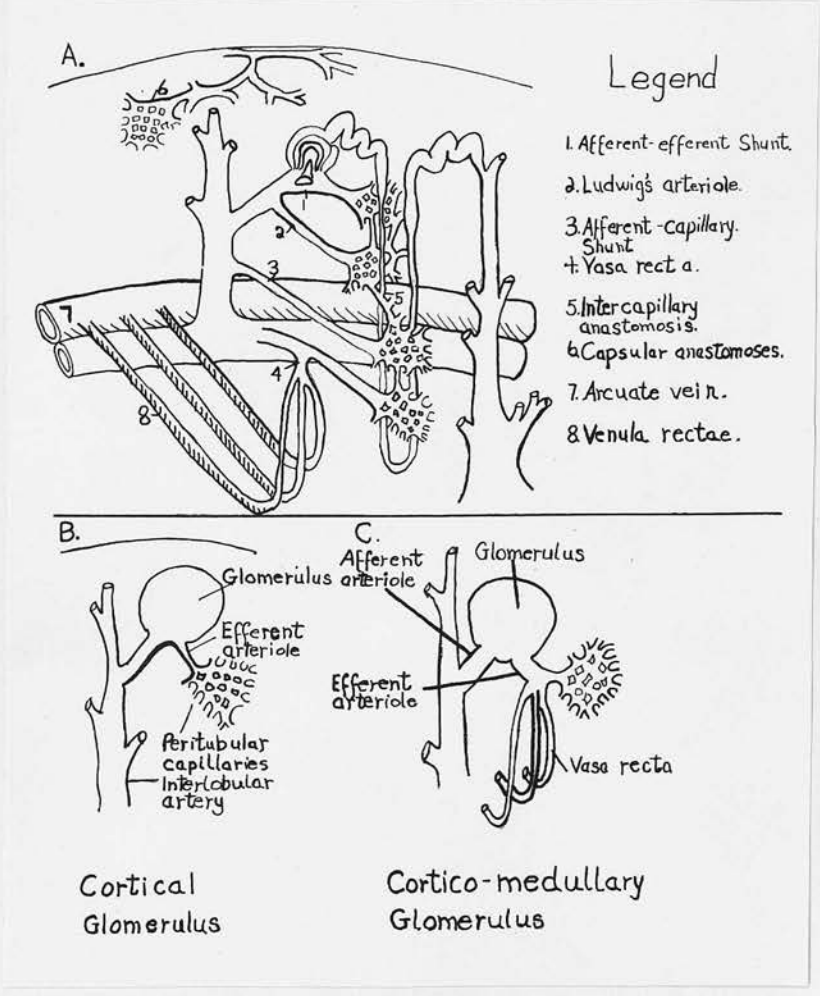
Fig.5.A.The hemisectioned human kidney in diagramatic fashion. (From Allen, A.C.; The Kidney, Medical and Surgical Diseases, New York, 1951, Grune and Stratton.)
B. Radioarteriograph of entire rat kidney showing the arterial tree outlined with bismuth contrast medium. Enlarged X 6. The vascular patterns are strikingly similar.

The nephron constitutes the architectural unit of the kidney and is diagramatically shown in relation to its immediate blood supply in figure 6 A. It comprises a glomerulus which ranges between 200 and 250 microns in diameter in the human and is formed by the invagination of the Malpighian tuft into Bowman's capsule; the proximal convoluted tubule which measures approximately 60 microns in diameter and 14 millimeters in length; the descending limb of Henle's loop with a diameter of 15 microns and a length of between 4 and 10 mms depending on the site (cortical or cortico-medullary respectively); the ascending limb of the Loop of Henle with a diameter of 30 microns and a length of 9 mms; the distal convoluted

tubule which measures 35 microns in diameter and 5 mms in length; and the collecting tubule with a length of approximately 20 mms and a diameter at the area cribrosa equal to roughly 100 microns. The total length of the nephron is approximately 55 mms in the human kidney, to which all the above measurements apply. It has been estimated that in both kidneys there is a total length of 75 miles of nephron.

Blood Supply. The renal arteries come off the aorta at an oblique angle. In the rodent they bifurcate at a point $\frac{2}{3}$s of the way along towards the kidney; in the human they enter the renal hilum before dividing into an anterior and a smaller posterior branch, as shown by the remarkable neoprene cast studies of More and Duff, 1951. These are "end-arteries", without anastomoses. From these, in the region of the pelvic fat, arise the interlobar arteries which extend into the columns of Bertin between the medullary pyramids of the human kidney. Vessels of comparable size arise within the renal hilum in the rat and extend in several directions to the cortico-medullary junction (figure 5 B). At the junction of medulla and cortex the vessels fan out in arterial arcs which constitute the arcuate arteries. The next order of arteries arise in vertical fashion from the arcuates and run into the thin cortical rim of the kidney as the interlobular arteries. From the minute

interlobular arteries arise the afferent arterioles. The course, from this level onwards is best traced by reference to figure 6. It is estimated that 90 percent of the blood normally flows through the glomeruli and that the remaining 10 percent bypasses the glomeruli through the mechanism of shunts such as shown in figure 6, A and C. (The potential shunt in figure 6 C, occurs only following degeneration of the glomerulus).



Err. Fig 6 A. 3 = Interlobular-capillary shunt.

Fig. 6. A. The vascular shunt pathways drawn in relation to the nephron, cortical variety.
B. A cortical glomerulus, showing the relatively large afferent arteriole and narrow efferent arteriole. Compare with Fig 6. C.
C. A cortico-medullary glomerulus, showing the efferent arteriole to be equal in diameter to the afferent arteriole and to contribute direct arterial pathways to the medulla.
(Drawn after Allen; The Kidney, Medical and Surgical Diseases. New York, 1951, Grune and Stratton.)

There would appear to exist five available pathways by which the 10 percent of arterial blood may reach the renal parenchyma without traversing the glomeruli: 1. Arteriolar shunt from the afferent to the efferent arteriole; 2. Ludwig's arteriole from the afferent arteriole to the tubules, and a corresponding arteriole from the interlobular branch; 3. Anastomoses between peritubular capillaries; 4. Collateral circulation from vessels penetrating the renal capsule; and 5. Arteriolae verae rectae from the interlobular and arcuate arteries to the medulla. Trueta and his associates (1947) consider the arteriolae verae rectae to be simply merged afferent-efferent arterioles consequent upon glomerular degeneration and thus to be of little practical importance as an extra-glomerular shunt mechanism.

The medulla is supplied largely by the arteriolae rectae spuriae, or vasa recta (figure 6 C.), which arise from the efferent arterioles of the cortico-medullary glomeruli and run into the medulla as parallel twigs. As indicated in figure 6, B and C, the cortical glomeruli have efferent arterioles of much smaller calibre than the corresponding afferent arterioles, whereas in the cortico-medullary glomeruli the efferent arterioles are as large or larger than the afferents (Trueta et al, 1947). In the present investigations, using the rat, the above differentiation is by no means hard and fast, though some glomeruli do follow this pattern.

The venous return takes origin from the capillaries of the outer cortex, which, by confluence, form the stellate veins. These, in turn, drain into radially arranged interlobular veins and the venous system then approximates its arterial counter-:part to emerge eventually as the renal vein. The medulla is drained via the venulae rectae into the arcuate veins.

Innervation. The nerve supply of the kidney, especially the distribution of its finer ramifications, has not been completely solved. Certainly the kidney

is well supplied with sympathetic fibres from the splanchnic nerves and with parasympathetic vagal fibres. On the other hand, explantation experiments in which the kidney is removed and re-inserted elsewhere in the circulation prove that normal renal function may occur in the absence of nerve control (Rhoads et al, 1934). It would seem, however, that neural influences are of some importance in certain pathological circumstances, notably in shock and other forms of stress. In the present investigation I have used ganglion blocking agents to counteract terminal neurigenic arteriospasm produced by the trauma of the injection technique. Improved filling resulted.

SECTION I

SECTION I

A morphologic investigation of the changes of progressive autolysis in human, rabbit and rat tissues.

The similarity of pre-mortem degenerative changes and post-mortem autolytic changes in the renal parenchyma is well recognized (cf. Mönninghof, 1939, and Allen, 1951). It became essential to distinguish between these types of changes in an investigation of the correlation of pre-mortem hepatic and renal damage in human autopsy material. Many excellent studies have been made, both on the morphological (Cruikshank, 1912, and Oka, 1920) and chemical aspects (Bradley, 1938), but it was not possible to apply the findings of these studies to the particular problem under consideration. The following studies were, therefore, directed to obtaining information necessary to the investigations noted above.

Materials and Methods

Materials include rabbit kidneys removed at nephrectomy and human and rat kidney and liver tissue obtained at autopsy. All blocks for histological study were removed in an identical fashion. They were excised

with a sharp knife using a minimum of pressure and immediately placed in an abundant amount of fixative. Following fixation they were dehydrated, cleared and embedded using identical schedules. Staining was carried out so that all sections to be compared were stained under identical circumstances. A survey was made of all routine stains with regard to selectivity in the detection of all stages of antemortem changes in the epithelia of the nephron from the earliest type of dysadaptation to frank necrosis. From this, the standard Hematoxylin and Eosin and the Masson Trichrome stains were chosen and employed throughout on 5 micron paraffin sections.

Procedure for Masson's Trichrome Stain

1. Fixation - Zenker-formal
2. Toluol I - 5 min.
3. Toluol II - 5 min.
4. Absolute Alcohol - 5 min.
5. 1% Iodine in Absolute Alcohol - 5 min.
6. Rinse in water.
7. 5% sodium thiosulphate - 5 min.
8. Wash in running water - 10 min.
9. Mordant in 5% ferric alum at 56°C for 5 min.
10. Rinse with distilled water.
11. Stain with Regaud's haematoxylin at 56°C. for 5 min.
12. 95% Alcohol - 5 min. (discard alcohol).
13. Fresh 95% Alcohol - 5 min. (move this Alcohol up to step 12 for use once there).
14. Differentiate with picric alcohol for 5 min. Discard picric alcohol.
15. Fresh picric alcohol for 5 min. (Move this picric alcohol up to step 14). By this time only nuclei should be stained.
16. Wash in running water for 10 min.

17. Stain in Ponceau-fuchsin for 5 min.
18. Rinse in 1% acetic acid water.
19. 1% phosphomolybdic acid for 5 min. (Discard stain).
20. Rinse with distilled water.
21. Stain with fast green for 5 min.
22. Rinse with distilled water.
23. Rinse with 1% acetic acid water.
24. Dehydrate rapidly with 3 changes of absolute alcohols. Each time discard the first one and move the other two up so the last alcohol is a fresh one.
25. Clear in 3 changes of toluols, 5 min. in each.
26. Mount in permount.

STOCK SOLUTION FOR ZENKER FIXATIVE

Potassium bichromate - 2.5 gm.
Mercuric Chloride - 7 gm.
Distilled water - 100 c.c.
For Zenker-formal use 80 c.c. of above and 20 c.c. of 40% formaldehyde.

STAINING SOLUTIONS FOR MASSON'S TRICHROME STAIN

Regauld's haematoxylin

Haematoxylin .....1 gm.
Alcohol (95%) .....10 c.c.
Glycerin .....10 c.c.
Water, distilled .....80 c.c.

Picric Alcohol

Alcohol 95% saturated with picric acid (about 7%) ..... 2 parts
Alcohol, 95% .... 1 part.

Ponceau-Fuchsin Solution

Acid fuchsin .....0.3 gm.
Ponceau de xylidene .....0.7 gm.
Water, distilled .....100 c.c.
Acetic acid, glacial .... 1 c.c.

Phosphomolybdic acid

Phosphomolybdic acid .1 gm.
Water, distilled ..100 c.c.

Fast Green

Acetic acid, glacial ..... 1 c.c.
Water, distilled .....100 c.c.
Fast green ..... 2 gm.

Experiment 1: Autolytic changes in the kidneys of nephrectomized rabbits.

Procedure. The left kidneys were removed under ether anesthesia from 10 apparently healthy adult rabbits of both sexes and average weight of 3 kgs, hemi-:sectioned by median longitudinal incision and placed in bottles containing N-saline for storage at room temperature (15°C) (Room temperature of 15°C was chosen in preference to body temperature in order to delay bacterial growth in the non-sterile tissues). Vertical sections were removed from each specimen, commencing from one pole and placed in 10% formalin at time intervals 0, 3, 7, 24, and 74 hours respectively. (10% formalin was employed in this portion of the study to conform with techniques in the human autopsy depart-:ment.)

Results. In H&E sections there is noted a pro-:gressive depletion of cytoplasmic and nuclear substances in the epithelium of the proximal convoluted tubules (figs. 7 to 11 inclusive). After 3 hours of autolysis there is no demonstrable change in the tissues. When 7 hrs have elapsed a definite tinctorial loss is noted in the eosinophilia of the cytoplasms and the basophilia of the nuclei. The luminal borders become more ragged

and the cells appear swollen. There is a suggestion of perinuclear vacuolization in the epithelium of the proximal tubules, while the glomeruli, distal and collect-:ing tubules appear unaffected as yet.

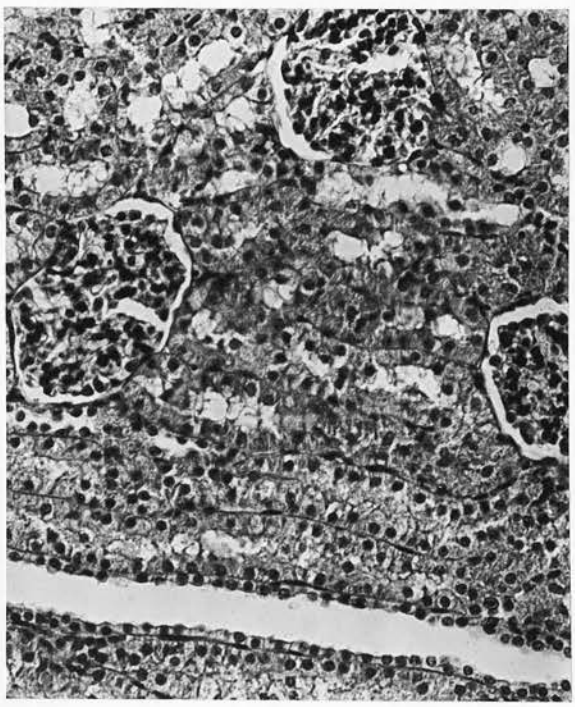


Fig. 7.
CR 5. Rabbit kidney. H&E x300. Control, no autolysis.

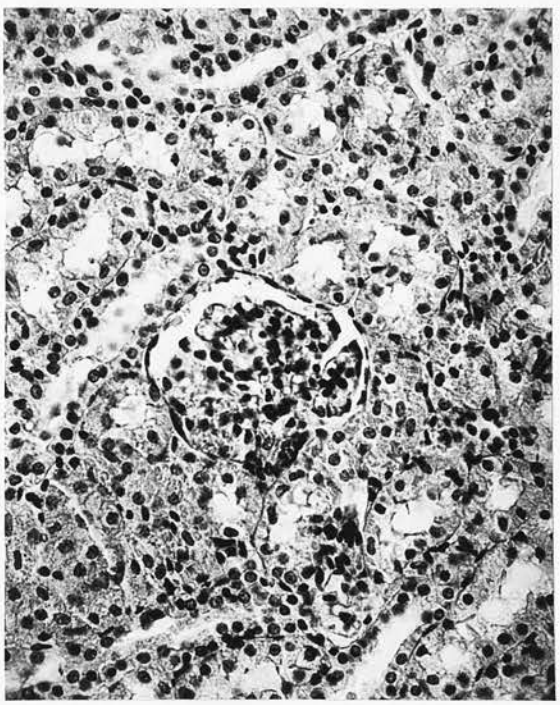


Fig.8.
CR 5. Rabbit kidney. H&E x300. 3 hrs autolysis: no perceptible changes are noted.

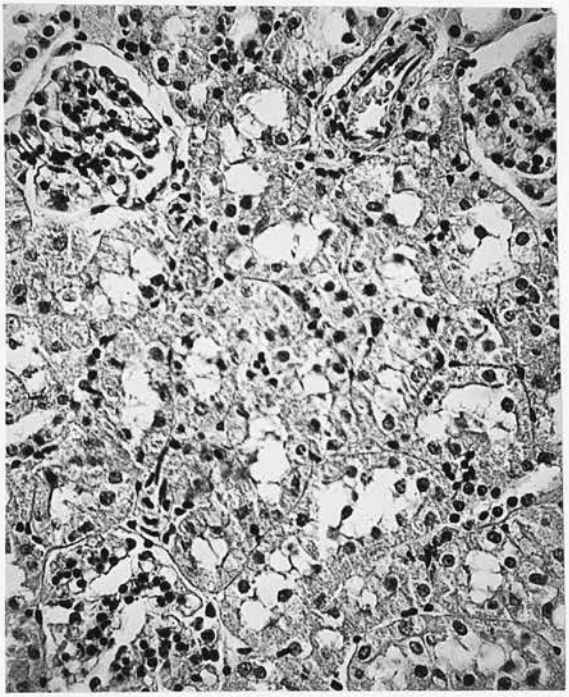


Fig. 9.
CR 5. Rabbit kidney. H&E x300. 7 hrs autolysis. Nuclear & cytoplasmic pallor in proximal tubules, with perinuclear halos.

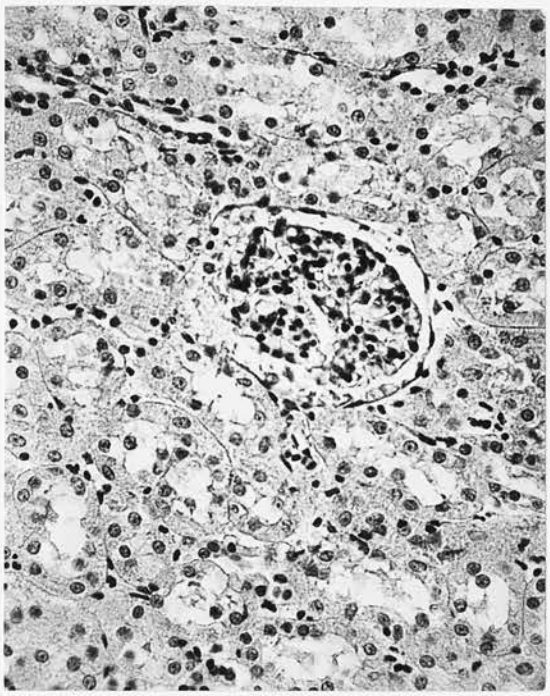


Fig. 10.
CR 5. Rabbit kidney. H&E x300. 24 hrs autolysis. Fluid & debris in various lumina. Some further tinc-:torial loss and commencing disruption in proximal tubules. Pyknosis of glom-:erular, interstitial & distal tubular nuclei.

In the 24 hr period, many further changes have developed. Cytolysis and karyolysis have progressed to a state of partial disruption of the proximal epithelium. Cells now appear showing detachment from their basement membranes, while the tubal lumina contain much eosino-:philic, granular and cellular debris. Many nuclei have disappeared or remain in shadow form. The glomeruli have also undergone a change, namely the addition of a granular, eosinophilic material which partially

obliterates the capsular space. In contrast to the surrounding tubal epithelium, the glomerular nuclei stand out in pyknotic relief. The interstitium is just commencing to show edematous distension, with formation of tiny clear spaces often containing a pale pink material. Nuclear pyknosis has now commenced in the epithelium of the distal portions of the nephron and the staining properties of the cytoplasm has changed from a faint to a pronounced acidophilia. The cells appear slightly more distended and the lumina contain variable amounts of principally a-cellular, granular, eosinophilic debris. After 74 hrs of autolysis little recognizable renal structure remains. The changes may best be summed up as persistence of the basement membranes of the entire nephron; loss of all nuclear and cellular structure in the proximal tubules and loops of Henle, its place being occupied by granular, eosinophilic debris; gross edema of the interstitium; and the rare persistence of some distorted nuclear and cellular detail in the glomeruli and distal nephron. Pyknosis, while rarely seen in the upper portion of the nephron, is a feature of the remaining nuclei of the glomeruli, distal and collecting tubules. In addition the cytoplasm of the distal tubules tends to become more densely eosinophilic.

Sleeve separation is always more pronounced in the distal than in the proximal tubules.

With the trichrome stain essentially similar changes are noted, the overall picture being that of progressive cyto-karyolysis and avulsion of the epithelium of the proximal convoluted tubules and descending loops of Henle, with increasing nuclear pyknosis and initial increase in cytoplasmic density, followed by a very slow cytoplasmic dissolution in the distal nephrons and glomeruli. The tubal lumina tend to accumulate a reddish, granular material which arises from lysed epithelium. Interstitial changes are those of progressive edema. These changes are well marked after 24 hrs and by 74 hrs the tissue is practically unrecognizable.

In three of the 10 rabbit kidneys, nuclear pyknosis and cytoplasmic degeneration in the epithelium of the proximal convoluted tubules and epithelial casts are observed in the control sections taken immediately after nephrectomy. As autolysis proceeds, these pre-mortem degenerative changes stand out in sharp contrast to autolysing normal intervening cells, with pyknosis and acidophilia of cytoplasm persisting until the stage of general dissolution at which point differentiation becomes impossible. (fig. 12, A & B.)

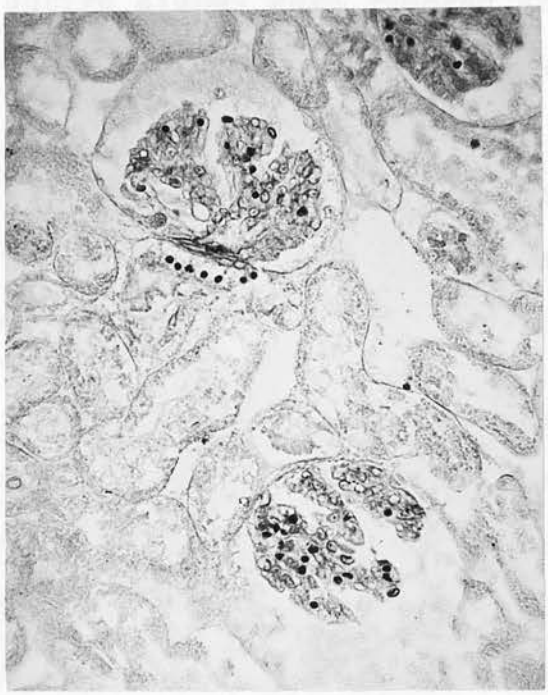
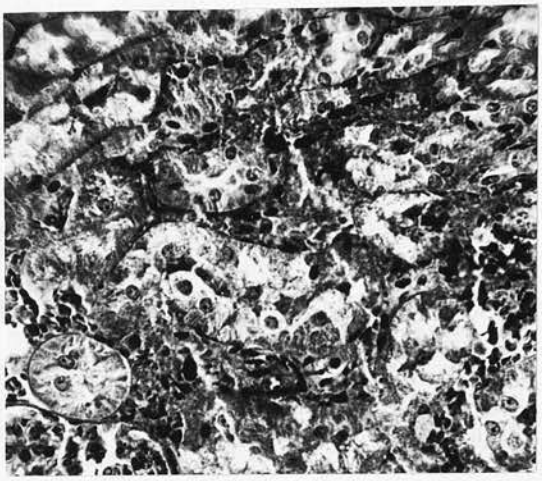


Fig. 11.
CR 5. Rabbit kidney. H&E x300. 74 hrs autolysis. Basement membranes persist throughout. Some pyknotic nuclei remain in the glomeruli and distal tub-:ules. Nuclear & cyto-:plasmic detail has dis-:appeared in the proximal tubules. Gross interstitial edema is present.

A



B

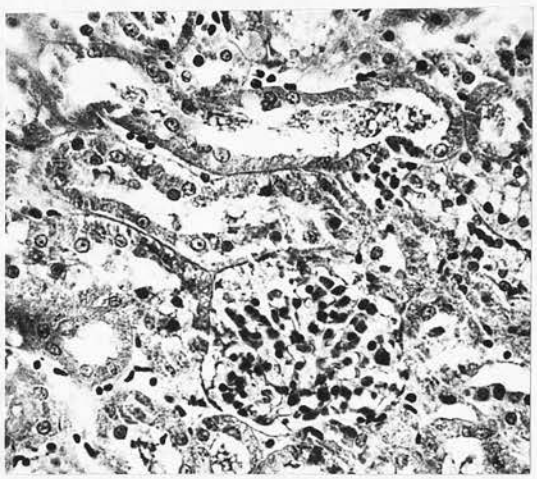


Fig. 12.
CR 1. Rabbit kidney. Trichrome x300. A. No autolysis. Many degenerating cells with pyk-:nosis & intense acidophilia are noted in the proximal tubules.

B. 24 hrs autolysis. The nuclear & cytoplasmic changes persist in easily recognized fashion.

Experiment 2: Autolytic changes in human kidney and liver tissue.

Procedure. A 500 gram block of liver and one entire kidney were removed at autopsy, blocks placed in 10% formalin, and the remaining tissue wrapped in towelling soaked in N-saline and allowed to lie at room temperature (15°C), with random samplings over variable intervals of time into 10% formalin. A total of 6 autopsy cases were studied in this fashion.

Results. The renal changes, observed over a wider range of time intervals, correspond closely with those seen in the rabbit kidneys. There is a lesser tendency to complete dissolution and to imbibition of fluids into capsular spaces and tubal lumina, presumably due to the drier conditions of storage. Again, as in the case of the rabbits, if pre-mortem changes are present in control sections they can be traced throughout the periods of autolysis, necrotic epithelial cells retaining the dense chromatophilia of cytoplasm and nucleus. The number of pyknotic nuclei in the proximal tubules appears to remain constant, while the total number of nuclei decreases. Such a case, A-487, is shown in figs.13, 14 and 15.

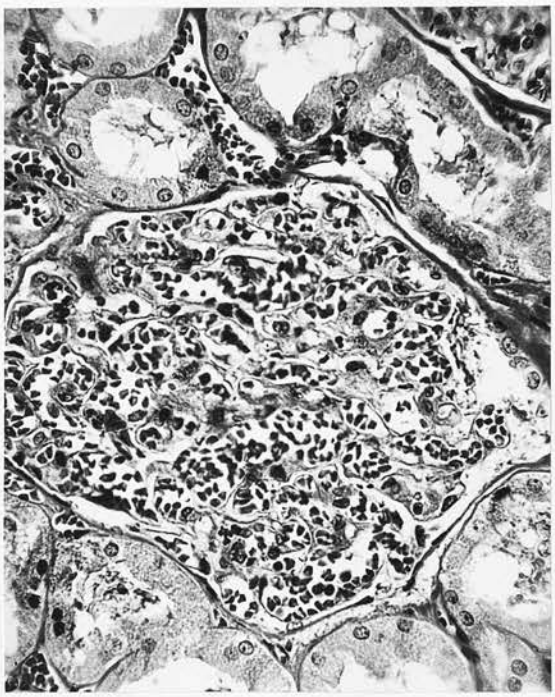


Fig. 13.
A.487. Human kidney.Trichrome x300. Control section taken 1½ hrs after death. Note scattered proximal epithel-:ial cells with pyknotic nuclei and hyperchromatic cytoplasms, plus casts.

Fig. 14.
A.487. Human kidney. Tri-:chrome x300. 27½ hrs autolysis. The pre-mortem nuclear and cytoplasmic changes are seen to persist, in the company of cyto-:karyolysis and fluid imbibition.

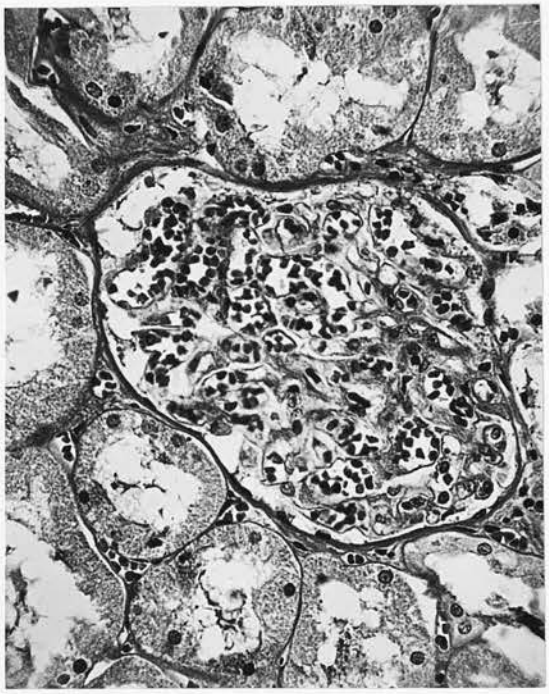


Fig. 15.
A.487. Human kidney. Tri-:chrome x300. 57½ hrs autolysis. The degener-:ative pre-mortem changes can still be identified in the proximal tubules. Active nuclear and cytoplasmic lysis is seen elsewhere. A small fragment of distal tubule appears at the right with densely pyknotic nuclei & dark-staining cytoplasm.

Fig.16.
A.487. Human liver. Tri-:chrome x300. control section, 1½ hrs post-mortem, showing sinusoidal con-:gestion.

The hepatic changes appear more variable. In four of the six livers there is noted chromatin condensation in the parenchymal nuclei, with an overall increase in the number of pyknotic and karyorrhectic nuclei. The remaining livers in this group reveal, instead, progressive karyolysis. Cytoplasmic changes are constant in all livers, consisting of shrinkage of total cell volume and increased staining intensity in the later phases of autolysis. There is minimal imbibition of fluid into the sinusoids and the branch-:ing pattern of the hepatic cords remains intact as late as 70 hrs post-mortem. (Figs. 16, 17, and 18, liver from the same case as in figs. 13, 14 and 15).

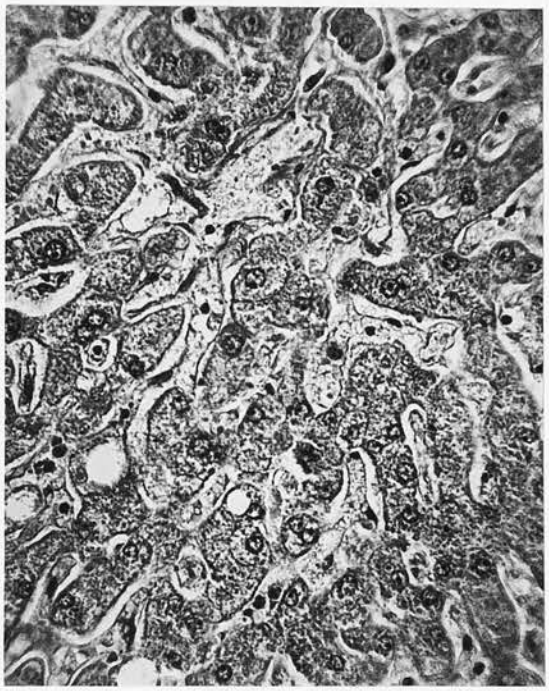


Fig. 17.
A.487. Human liver. Tri-:chrome x300. 27½ hrs autolysis. An increase of fluid and amorphous debris in the sinusoids and a slight coarsening of the granularity of the cyto-:plasm are the only note-:worthy features at this period.

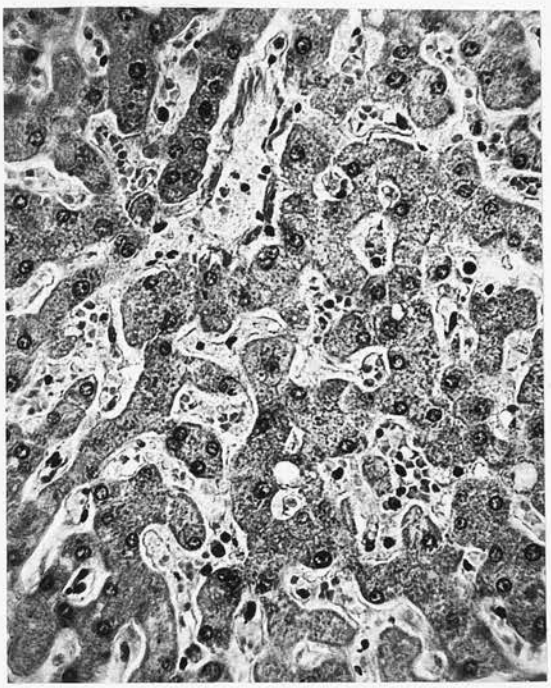


Fig. 18.
A.487. Human liver. Tri-:chrome x300. 57½ hrs autolysis. There is a slight shrinkage of the cell volume and an increase in the number of pyknotic nuclei. The cytoplasm of the parenchymal cell has taken on a much greater staining intensity.

Experiment 3: Autolytic changes in rat kidney and liver tissue.

A. Procedure. Ten healthy Wistar strain albino rats of both sexes and average weight of 175 grams were killed by excising the kidneys and liver under ether anesthesia. Blocks of kidney and liver were placed immediately in Helly's fluid and one kidney and a 3 gram

portion of liver were placed in N-saline and allowed to autolyse at room temperature (15°C). Samples were fixed in Helly's fluid at 0, 20, 44 and 96 hour intervals. In addition to the routine H&E and trichrome stains, sections from two rats were followed by the periodic acid - Schiff stain (according to McManus, 1948).

Results. The renal changes parallel those of the rabbit kidneys, but a brownish, granular material tends to concentrate at the basal edge of the proximal tubular epithelium in the 20 hr specimen, later to disappear completely. (Figs. 19, 20, 21 and 22).

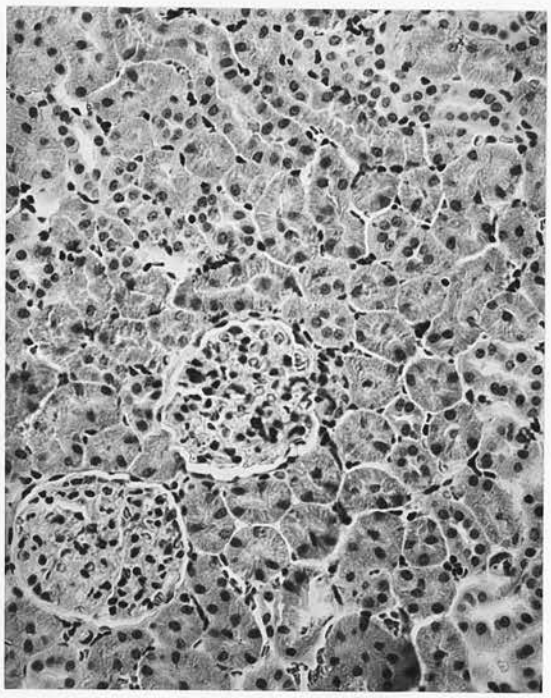


Fig. 19.
C 4. Rat kidney. H&E x300.
Control, no autolysis.

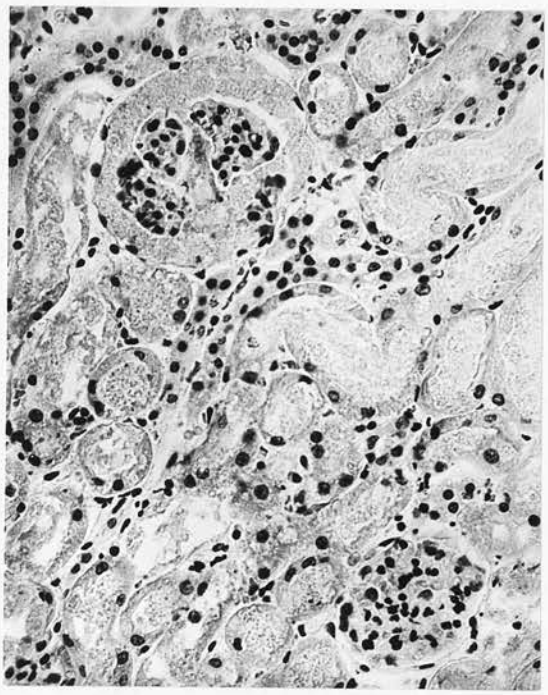


Fig. 20.
C 4. Rat kidney. H&E x300.
20 hrs autolysis. All lumina are distended with a debris-laden fluid. There is much evidence of cyto-karyolysis. The number of pyknotic nuclei in the identifiable proximal tubules appears identical with that of the control section. Pyknosis is mainly confined to glomeruli and distal tubules. Much interstitial edema is seen.

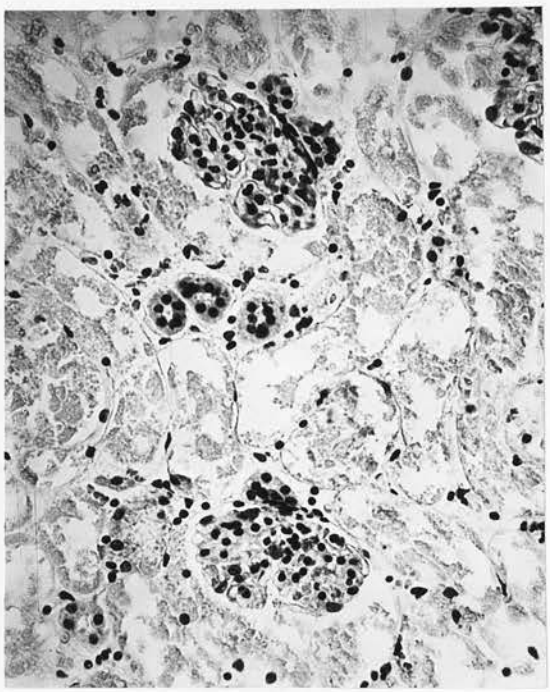


Fig. 21.
C 4. Rat kidney. H&E x300. 44 hrs autolysis. Cytokaryolysis has been completed in the proximal tubules leaving only granular debris. Distal tubular, interstitial and glomerular nuclei remain pyknotic and the cells are relatively intact, though separated from their basement membranes.

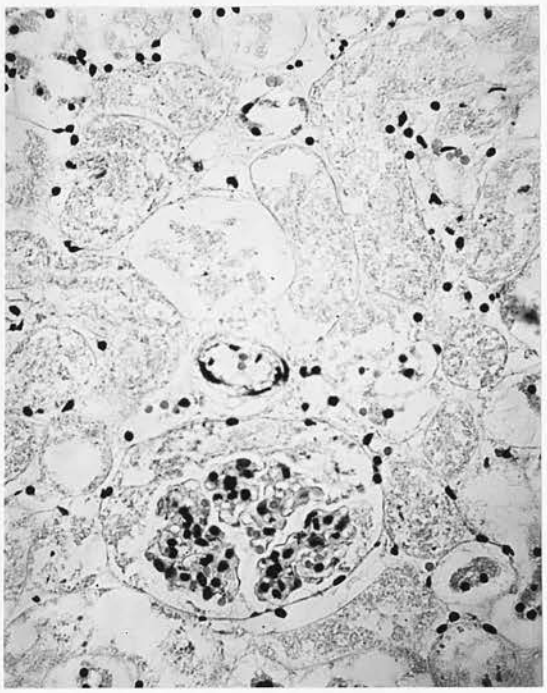


Fig. 22.
C 4. Rat kidney. H&E x300. 96 hrs autolysis. Basement membranes, pyknotic glomerular, interstitial and rare distal tubular nuclei are all that remain of recognizable renal tissue.

The hepatic changes consist of progressive shrinkage of the cord cells evenly throughout the lobule, plus fluid imbibition into sinusoids and the gradual accumulation therein of an amorphous eosinophilic material. The cytoplasm of the parenchymal cell during the first 20 hrs of autolysis reveals increased pallor of staining and

the nuclei appear to be undergoing karyolysis. A rather striking reversal of nuclear and cytoplasmic staining is noted beyond this time interval, consisting of a decided increase in density of the cytoplasmic contents displaying a peculiar "ground glass" granu-:larity, associated with a minor decrease in the cell volumes and a darkening of nuclear chromatin structure in the intact nuclei. The latter approaches pyknosis and/or karyorrhexis in several instances. (Figs. 23, 24, 25 and 26). There is no apparent accumulation of stainable fluid in the spaces of Disse. Sections stained by P.A.S. reveal poly-saccharide material in the 0 and 20 hr specimens, but are negative beyond 20 hrs.

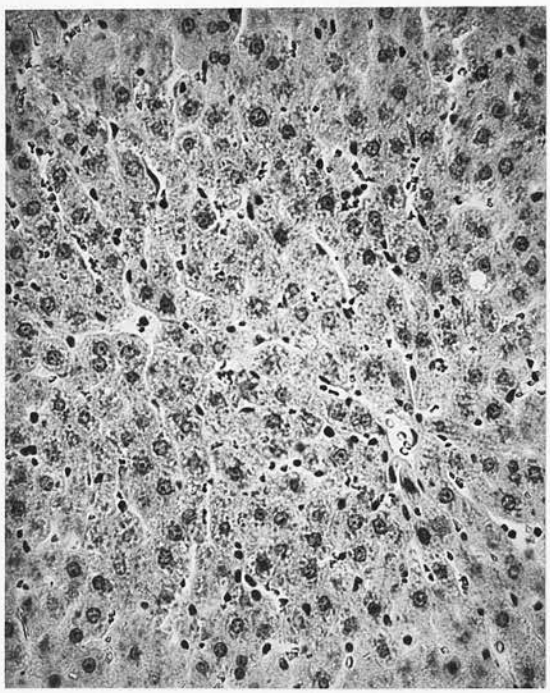


Fig.23.
C 4. Rat liver. H&E x300.
Control, no autolysis.

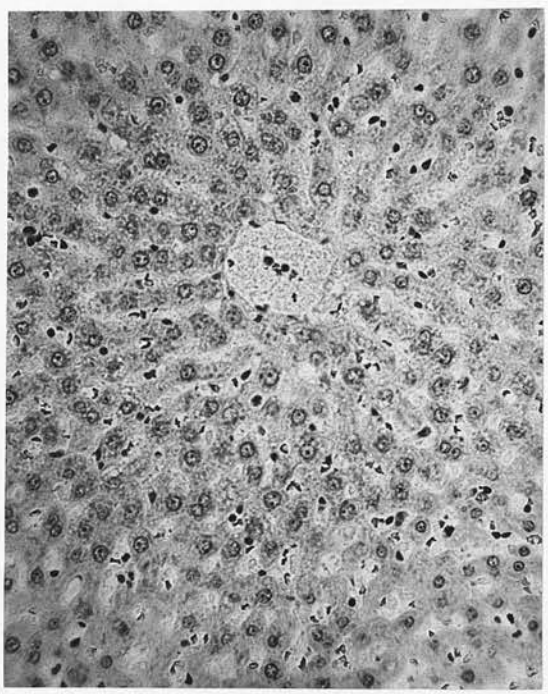


Fig.24.
C 4. Rat liver. H&E x300.
20 hrs autolysis.There is a slight shrinkage of the cord cells plus generalized cyto-:karyolysis and imbibition of fluid into the sinusoids.

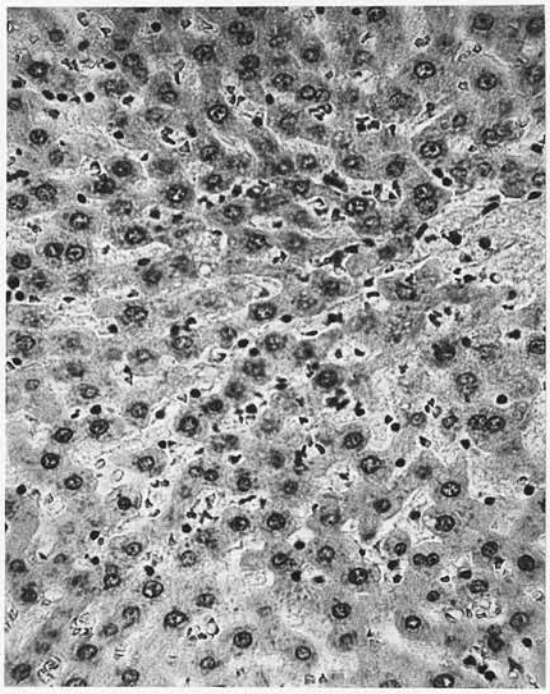


Fig. 25.
C 4. Rat liver. H&E x300. 44 hrs autolysis. Increased density of the nuclei and cytoplasms appears in striking contrast to Fig.24. The change is likened to "ground glass".

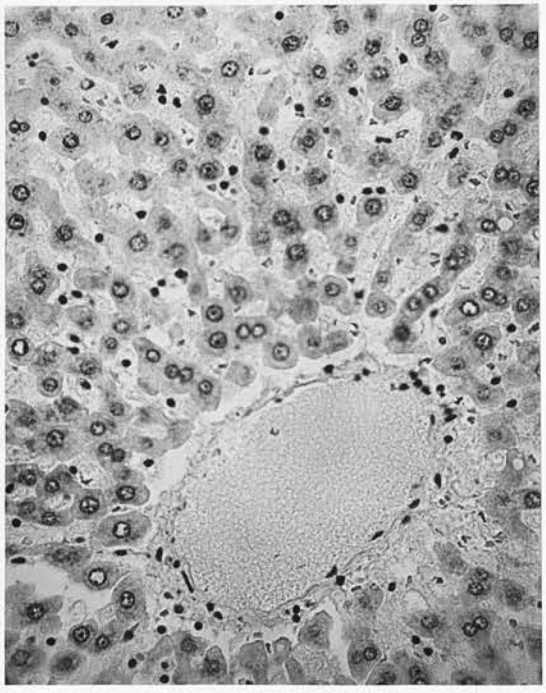


Fig. 26.
C 4. Rat liver. H&E x300. 96 hrs autolysis. Pyknosis and karyorrhexis is seen in a few nuclei and the density of the cytoplasms remains unchanged. Despite marked sinusoidal distension the lobular structure remains intact, with no disruption of the cords.

B. Procedure. Four 300 gram male Wistar rats were killed by an overdose of ether and the right kidneys and a small portion of the livers were excised as controls through small right lateral incisions with a minimum of manipulation. The holes were plugged with absorbent cotton and the carcasses allowed to lie at room temperature (15°C), one being chosen on each successive day from which the left kidney and a further

portion of liver tissue were excised. Blocks of all tissue were fixed immediately in Helly's fluid. The investigations thus covered periods of 0, 24, 48, 72 and 96 hours.

Results. All rats reveal a slight degree of intrinsic renal disease, as evidenced by an unusually large number of degenerative cells in the distal and proximal convoluted tubules of control sections of the kidneys. The lesions are most marked in the rat employed for the 72 hr specimen, where protein exudate is found in the capsular spaces and tubal lumina in addition to acute degenerative changes in the epithelial cells.

The autolytic picture in H&E sections follows closely on the picture seen in saline autolysis with a few important alterations dependent upon moisture content and bacterial contamination. All former features are present, but on a reduced scale. The progress of autolysis is much delayed, fluid imbibition into all types of lumina is minimal and the initial brownish, granular change in the basal portions of the epithelia is more intense, lasting for 48 hours. After this period, however, cyto-karyolysis progresses actively in the cortical regions, with loss of all cellular

structure in the proximal tubules and disruption and nuclear pyknosis in the distal tubules and glomerular tufts. In small patches of superficial cortex and in the cortico-medullary zone there is partial persistence of nuclear and cellular detail in all components of the nephron as late as 96 hrs. In sections stained by Masson's Trichrome, pre-mortem cytoplasmic degenerative changes can be recognized as late as 48 hrs post-mortem after which, the bright red smudgy or granular pigment becomes progressively browner and non-specific. The pyknotic nuclei, however, persist into the stage of general dissolution. At no stage does the autolytic process in the proximal tubules mimic pre-mortem changes.

In the liver the changes are identical with those of saline autolysis apart from the relatively slight amount of fluid which is imbibed into the sinusoids.

Discussion

It is essential in the modern investigation of disease to be able to differentiate between pre-mortem morphological changes of any degree and post-mortem changes, in order to correlate structural alterations with the newer knowledge of physiological alterations in disease. In a study of post-mortem changes in the parenchymatous organs of man, it is not possible to take

serial sections from the organs over any great period of time while leaving them in their natural environment. For the purpose of investigating morphological post-mortem autolytic changes it is essential to have initial control sections as a starting point for the comparison of later post-mortem changes. It seemed the only feasible method of experimentation for such a study was to remove the organs from the body, taking control material immediately on removing the organ and then removing further blocks for study while keeping the organ at moderate temperatures, moist and free of gross contamination. To compare with these changes in man, it seemed reasonable to study animal tissues under the same circumstances. In all these experiments, in trying to relate the post-mortem changes under the conditions of the above experiments with post-mortem autolysis as it occurs in the parenchymatous organs of the body, one must assume that the quality of the post-mortem changes is identical. This assumption seems valid in comparing changes seen in rats in experiment 3A with the changes in experiment 3B. In all of these experiments the rate of change was not considered important because we are ignorant of all the conditions which determine the speed of post-mortem autolysis in man. For this

reason, it was assumed that in order to differentiate pre- and post-mortem changes, one must look for qualitative differences. In this regard, the early work of Cruikshank (loc. cit.) demonstrating the striking effect of bacteria on the autolytic process, influenced my choice of a non-sterile environment such as exists under natural conditions. In a consideration of the results of this work, it must be borne in mind that post-mortem changes not only occur in all normal tissues but that post-mortem changes are superimposed on any pre-mortem changes which may be present.

In the above experiments it was possible to denote, in the kidneys, qualitative differences between purely post-mortem alterations and alterations that consisted of pre-mortem changes plus whatever post-mortem autolysis would occur after the control section was taken. These differences were reported in the following manner. Pure post-mortem alterations of proximal convoluted renal tubules followed the pattern of cyto-karyolysis, a progressive depletion of cytoplasmic and nuclear contents. This change was modified in the case of the rat by a preliminary basal concentration of brownish pigment in the cytoplasm which subsequently dissolved away. (A similar pigment is often seen in autolytic

human kidneys, but failed to manifest itself in the cases chosen for this study). However, in tissues where the control sections showed pre-mortem changes of nuclear pyknosis plus an increased density and eosinophilia of the cytoplasm, these changes persisted and were accentuated by post-mortem autolysis into the later stages of lysis. In the distal convoluted tubules, on the other hand, the normal course of post-mortem autolysis was nuclear pyknosis and increased density and eosinophilia of the cytoplasm. But in those tissues where the control sections obtained at the time of death showed patchy similar changes interpreted as pre-mortem, these changes remained in advance to the similar qualitative post-mortem changes of the distal convoluted tubules for some time.

From these observations it seems possible to conclude that pyknosis and density with acidophilia of the proximal convoluted tubules always represents a pre-mortem change. On the other hand, in the distal convoluted tubules, nuclear pyknosis and acidophilic density of the cytoplasm is the normal first stage of post-mortem autolysis and pre-mortem changes can only be diagnosed by the presence of patchy areas showing this change well in advance of the general alterations. In assessing the qualitative differences between

ante-mortem degeneration and post-mortem autolysis, the Masson Trichrome stain is both selective and indispensable.

The autolytic changes noted in the majority of the human and all the rat livers followed the sequence of initial cyto-karyolysis and subsequent increase in density of nucleus and cytoplasm. At first the nucleus and cytoplasm of the parenchymal cell grew paler and more indistinct, and there was a perceptible shrinkage of the cell volume. Later the cytoplasm increased in density and chromatin condensation occurred in the nuclei resulting in a hardened "ground glass" appearance. This alteration was found to coincide with a disappearance of glycogen as estimated by the periodic acid-Schiff reaction so that the shrinkage in cell volume is interpreted to be partially a result of glycolysis. In this regard Morrione and Mamelok (1952) have demonstrated the persistence of minute but stainable quantities of glycogen in human livers up to 48 hrs post-mortem. Throughout the entire period of autolysis there was a progressive accumulation of amorphous debris and fluid in the sinusoids, but even after 96 hrs immersion in saline no disruption of the branching pattern of the lobular cords occurred. On the other hand, van Beek and Haex (1948) have demonstrated

progressive post-mortem lobular disruption in a case of subacute hepatic necrosis, suggesting that autolysis may disrupt liver lobules which are the seat of prior necrosis. From the findings of the present series it is impossible to state whether or not lobular disruption can occur as a purely autolytic phenomenon, though I favor the view that it may do so. From the above observations it is apparent that post-mortem changes will tend to augment the histological picture of a wide variety of hepatic lesions such as necrosis, hepatitis, chronic passive congestion and the like, making accurate assessment of structural pre-mortem changes difficult.

Summary

1. The histological features of post-mortem autolysis under non-sterile, room-temperature conditions have been studied in the human and rat kidney and liver and in the rabbit kidney. The human tissues were autolysed in saline-moistened towelling; the animal tissues were immersed in N-saline or left 'in-situ' in the carcass.

2. The autolytic picture in the proximal convoluted tubules differs from that in the remainder of the nephron. In the former site the process is one of

cyto-karyolysis, while in the latter site acidophilic density of the cytoplasm occurs along with pyknosis of the nuclei.

3. The influence of conditions of storage produces only minor qualitative changes in the autolytic process, related to fluid and bacterial action. The quantitative effect is marked.

4. Ante-mortem degenerative changes in the proximal convoluted tubules are readily distinguishable from the changes of post-mortem autolysis by the presence of nuclear pyknosis and increased cytoplasmic staining intensity. These features are most pronounced with the use of Masson's Trichrome stain, but are lost with the disappearance of nuclear and cellular structure.

5. In the rat liver during the first 20 hours of auto-:lysis there is noted progressively increased pallor of staining in the cytoplasm and nucleus of the parenchymal cell and a slight shrinkage in volume. After 44 hours, however, the cord cells assume an increased intensity of stain in both cytoplasm and nucleus. The nuclear changes may subsequently progress to pyknosis and karyorrhexis. The biphasic nature of the changes in the hepatic cord cell would appear to be related to the glycogen content of the cell. The changes in human liver tissue are similar in the majority of specimens.

SECTION II

SECTION II

Incidence and Morphology of Associated Kidney and Liver Lesions in Human Autopsy Material.

Since Bywaters' description of the 'crush syndrome' and acute renal failure, there has been much interest in the kidneys of acute renal failure which accompany a broad spectrum of clinical entities, (Beall et al, 1941; Bywaters and Beall, 1941; Bywaters, 1942; Bywaters and Dible, 1942; Bywaters and Dible 1943; Lucke, 1946; French, 1950; Oliver et al, 1951; and Bull and Dible, 1953). A select group of cases in which acute oliguria-anuria accompanies hepatic and renal damage within rather poorly defined limits has been referred to as the 'hepato-renal syndrome'. In addition, there are many reports of infections, chemical poisons, allergies, etc., leading to death from renal failure in which are associated lesions of both kidneys and liver. Some recent autopsy cases revealed damage in both livers and kidneys in the absence of the clinical picture of the hepato-renal syndrome. In view of this it seemed desirable to ascertain whether any specific pathological process in the kidneys could be found in cases with varying types of hepatic disorders and to determine the exact nature of the renal lesion, where present.

The following study demonstrated the absolute correlation between a lesion in the kidney which I have designated as 'glomerulotubular nephrosis' and lesions of varying types in the liver.

Materials and Methods.

Cases with correlation of hepatic and renal lesions were obtained from the autopsy service of the Kingston General Hospital and chosen from two separate periods. The current autopsy material during the period from July 1st 1951 to May 31st 1952 (A-324 to A-523) was reviewed in its entirety, cases being chosen from those with renal or hepatic alterations. In addition, the autopsy files were studied for the year 1948 (A-1 to A-176), cases being selected from entries on the final anatomical diagnoses which implicated the kidneys, the liver, or both. A total of 50 cases were thus accumulated on the basis of showing renal and/or hepatic changes. All of these underwent refrigeration if the post-mortem was performed more than one hour after death.

In making the final selection, the following criteria were rigidly adhered to: all cases with renal disease such as glomerulonephritis, pyelonephritis,

nephrolithiasis, obstructive uropathy, arteriolo-:nephrosclerosis, marked arteriosclerotic scarring, polycystic and other developmental defects and neoplasm were excluded. In addition, post-mortem autolysis, when of sufficient severity to obscure ante-mortem change, was used as a ground for rejection. The renal lesions of the selected cases of non-specific combined glomerular and tubular damage included 5 cases of 'lower nephron nephrosis' (diagnosed on clinical and morphological grounds), 8 cases of nephrosis of varying etiology (eg. bile, toxic, etc.) and 37 cases of early 'non-specific nephrosis'. Using the above criteria, it was not found necessary to exclude any case of nephrosis due to lack of hepatic damage, and vice versa.

To control the selected material, it was necessary to determine whether renal lesions of the type present in the above group ever occur in the absence of hepatic disorders. It was also imperative to be able to assess the effect of early autolytic changes on both normal and abnormal kidneys and livers, as reported in Section I. The 15 cases constituting the controls were gleaned from the files of the following institutions: Kingston General Hospital, 3; Kingston Hotel Dieu Hospital, 1; Toronto Medico-legal department, 1; Edinburgh Royal Hospital for Sick Children, 5; other regional hospitals

of the S.E. Area, 5. (The presence of refrigeration facilities for the control group is noted in Table 2, appendix). In all instances, paraffin sections were cut at 5 microns and stained with hematoxylin and eosin and Masson's trichrome stains. 10% formalin was employed as the routine fixative throughout this study.

Results.

General Clinical and Pathological Findings.

The clinical and pathological findings and correlations of the selected and control groups are presented in Tables 1 & 2 respectively (appendix). In the group that showed hepatic and renal lesions, shock was estimated, when possible, from the recorded blood-pressures. If such were not available, the terminal history was assessed with regard to the probability of shock, such cases being recorded as '?' in the table. The incidence of shock on this basis is 24 % of the total group.

The following assessment of the absolute incidence of clinical diseases accompanying the hepatic and renal lesions is derived from the 'primary disease' and 'associated diseases' columns of Table 1 (appendix). It will be readily appreciated that these figures include several cases with more than one clinical disease,

which alters the values for relative incidence. Cirrhosis of the liver, congestive heart failure and malignancies each account for 26% of the total, the latter being present obstructively in the biliary tract in only 8% of cases. Peritonitis is found in 14% and bronchopneumonia, alcoholism and cerebral damage each account for 8%. The remainder is composed of a wide variety of entities; e.g. burns, rupture of esophageal varices, duodenal ulcer and its complications, pulmonary tuberculosis, Addison's disease, acute pancreatitis, infectious hepatitis, major abdominal surgery, etc. Obviously, few of these entities would fit the 'classical' picture of the hepato-renal syndrome, vaguely defined as 'hepatic necrosis plus acute renal failure related pathogenetically to surgical (and traumatic) intervention in the biliary and gastrointestinal tracts, liver and thyroid'.

At autopsy, there are found no constant gross characteristic features in the kidneys. The average combined weight of these organs is well within normal limits, with 10 (22% of adults) weighing over 400 gms and 6 (13% of adults) weighing under 250 gms. On the other hand, most livers show some abnormal macroscopic features; e.g. cirrhosis, acute yellow atrophy, severe chronic passive congestion, tumor, etc. The majority

of the livers, (30, or 66% of adults) are increased in weight above 1400 gms, while 6, (13% of adults) are below 1200 gms. These figures relate only to the adult segment of the selected population, which comprises 92% of the total. The ages range from 2 days to 92 years, the mode being 50 years.

The histopathology of the liver is recorded in Table 1 (appendix). Chronic passive congestion of the liver is a feature of 44% of the total and is co-existant with shock in 6%. Necrosis of the liver occurs in 56% of cases, distributed centrolobularly in 32%, focally in 22% and massively in one case (2%). Other hepatic lesions include severe fatty metamorphosis in 18%, bile retention in 12%, diffuse fibrosis (cirrhosis) in 26%, plus other disturbances of minor incidence: acute edema, cholangiohepatitis, leukemic infiltration, "cloudy swelling", and metastatic tumor.

Renal lesions of the selected group designated glomerulotubular nephrosis consist of degenerative changes in the glomerulus and all parts of the nephron and are present without exception in every case of the selected series. The detailed morphological features of this lesion will be described below. In addition,

under the heading 'additional renal disease' in Tables 1 & 2 (appendix), lesions other than glomerulotubular nephrosis, e.g. mild arteriosclerosis, bile nephrosis, etc., are recorded directly from the autopsy protocol.

In the control series, minor ante-mortem changes occur in 67% of livers and 27% of kidneys and include terminal hyperemia and minimal fatty metamorphosis of the liver and hyperemia of the kidney. In the kidney, the total picture of degenerative changes could not be interpreted as glomerulotubular nephrosis. Figures 27 and 28 are examples of normal kidney and liver respectively, from case No.11, Table 2 (appendix).

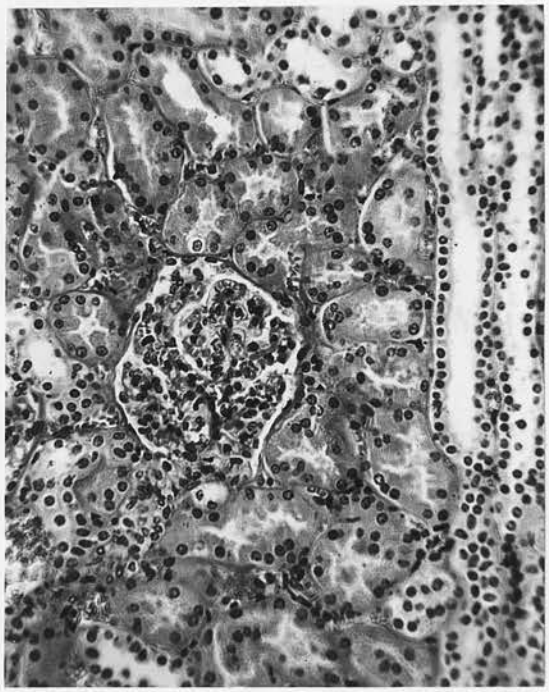


Fig.27.LHA-582. Case No.11.H&E x300.Normal kidney.

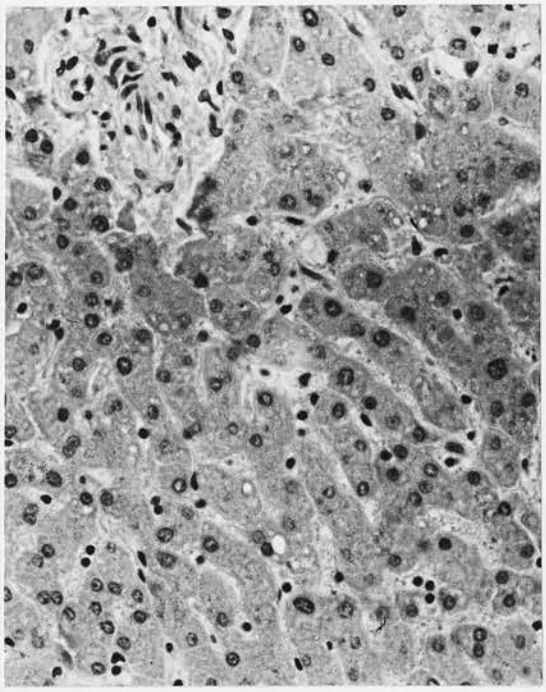


Fig.28.LHA-582.Case No.11. H&E x300.Normal liver showing a very slight degree of fatty change.

Microscopic Characteristics of Glomerulotubular Nephrosis.

From Table 3 (appendix) the following lesion in the nephron may be constructed. Glomerular changes: are those of protein exudate and capsular epithelial desquamation in the majority of capsular spaces plus varying degrees of swelling of the capsular epithelial cells. (Exudate and epithelial desquamation were also found, albeit in far slighter degree, in roughly 50% of the control kidneys). Most of these features are well displayed in Figs. 29, 30 and 39.

Proximal convoluted tubular changes: are those of slight to moderate luminal dilatation plus varying degrees of acute degeneration of the epithelial cells from nuclear pyknosis and "coagulative" hyper-chromaticity (bright, smudgy or more rarely corpuscular, red with the trichrome stain) of the cytoplasm, to shedding of the necrotic cells with the formation of mixed cellular and amorphous casts. (The latter are usually numerically small but almost invariably present). All changes show a patchy distribution, frequently more pronounced in the boundary zone between cortex and medulla. The presence of bile tends to decrease the nuclear staining intensity to a moderate degree. The changes in the proximal tubules are well illustrated in figures 29, 30 and 32.

(The hepatic lesions accompanying these cases are shown in figs. 31 and 33).

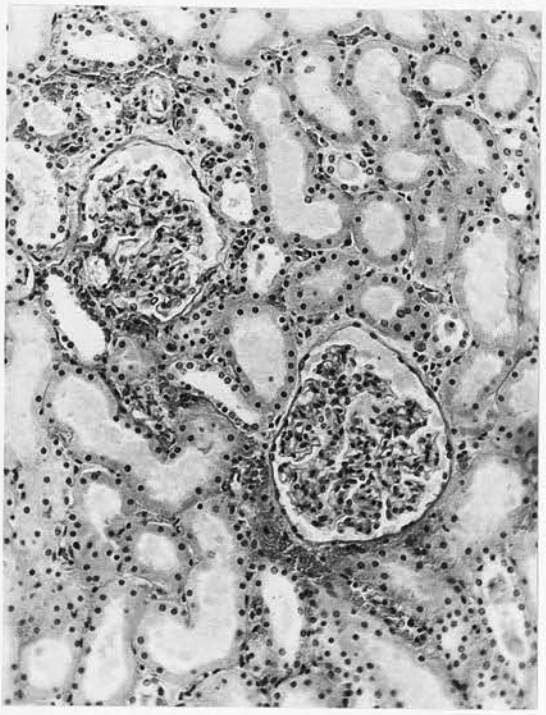


Fig.29. A-122-48, Case No.20. H&E, x150. Kidney in typhoid fever. Marked dilatation of proximal tubules, with protein and cellular material in lumina & capsular spaces and wide-:spread nuclear pyknosis. The thin limbs of Henle's loop contain hyaline casts.

Fig.30. A-147-49. Un-:listed. Trichrome, x150. Kidney from another case of typhoid. The acute focal lesions situated in the distal and proximal tubules stand out with this stain.

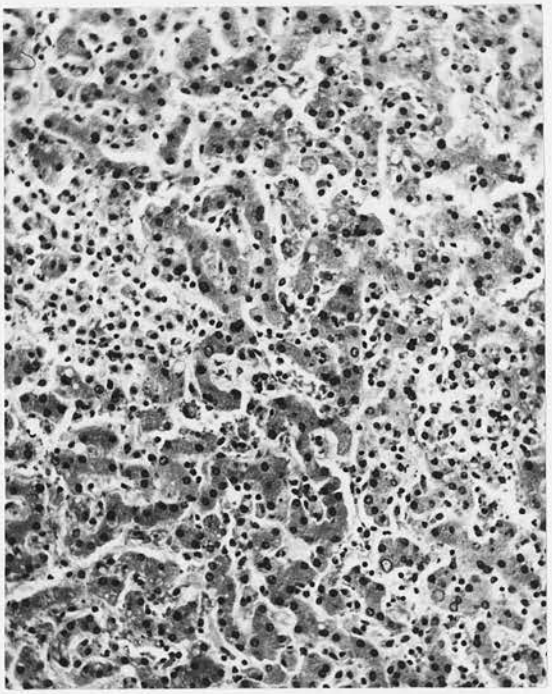


Fig.31. A-122-48, Case No.20. H&E x150. Liver from the case of typhoid shown in Fig.29, displaying the characteristic focal necrosis.

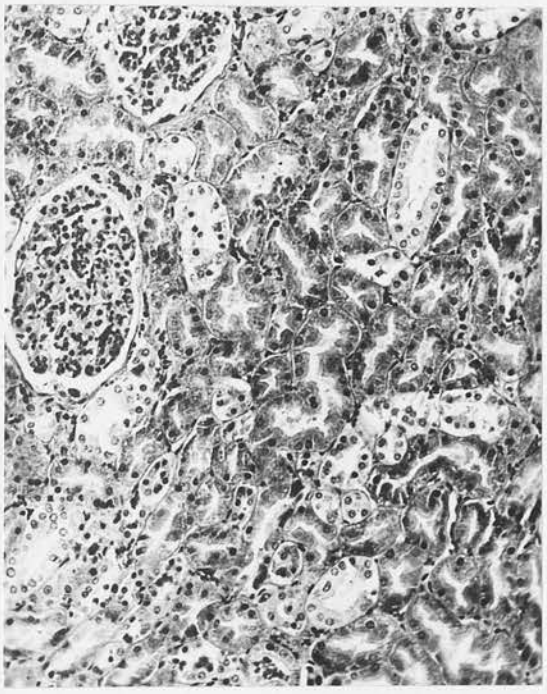


Fig.32. A-373, Case No.30. Trichrome x150. Kidney from a case of post-operative massive atelectasis, showing earliest of acute changes. Note nuclear pyknosis and coagulative changes in patchily distributed epithelial cells.

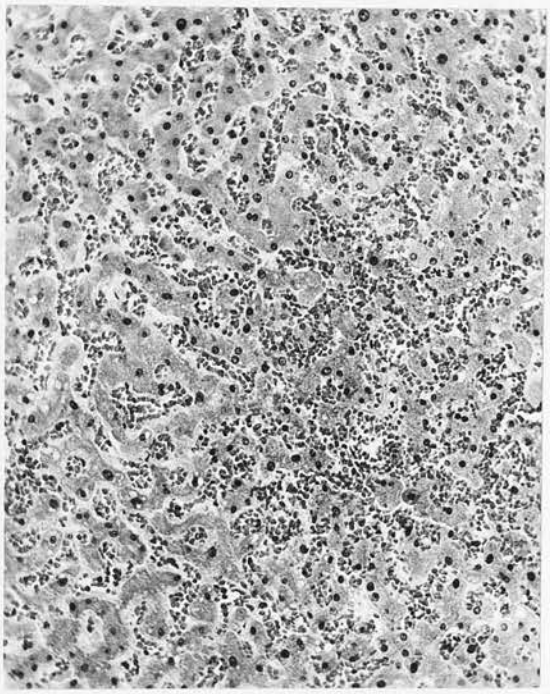


Fig. 33. A-373, Case No.30. Trichrome, x150. Liver from same case as fig. 32, showing acute and chronic passive congestion.

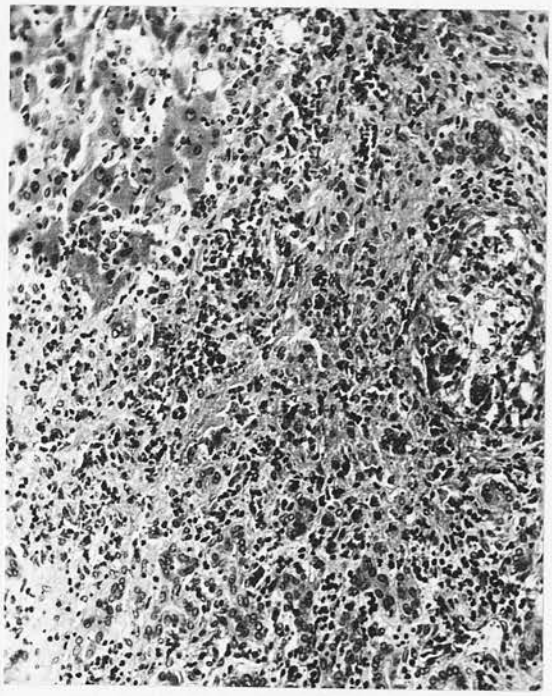


Fig.34. A-394, Case No.31. Trichrome, x150. Liver from a case of massive hepatic necrosis showing extensive parenchymal collapse and acute necrosis.

Descending limb of Henle's loop: shows changes reflecting the increased permeability of the glomerular filter, namely hyaline casts, composed of inspissated protein material. (These casts stain a pale green with Masson's trichrome stain).

Distal convoluted tubular changes (including the ascending limb of Henle's loop): are those of luminal dilatation, the tubules containing a wide variety of formed casts, including cellular, granular, hyaline and pigmented types; atrophic thinning of the epithelial

lining surrounding the larger casts; and patchy acute degeneration of the epithelium and simple nuclear pyknosis and epithelial sloughing which is indistinguishable from post-mortem autolysis. (The presence of a few large, hyaline casts, usually containing some orange-brown pigment, is a 'normal' feature of the adult human kidney, but if present in large numbers, especially if mixed with granular pigmented casts, they may signify glomerulotubular nephrosis in its more protracted forms). Fatty vacuolization is present in 50% of those selected cases in which the case protocol defines an etiologic toxic factor, but is negligible in the other varieties. Similarly, bile pigment is found only in the cholemic type of nephrosis. Tubal rupture and regeneration is seen primarily in the cases diagnosed on the protocol as 'lower nephron nephrosis' and is also noted in the case of massive hepatic necrosis. (The accompanying interstitial reaction is illustrated in fig. 36.).

Interstitial changes: are primarily those of edema. Inflammatory reactions are seen only in the more protracted lesions.

Vascular changes: are again rather specific to the protracted forms of nephrosis, angiitis being present in 60% of cases of 'lower nephron nephrosis' and in the case of cholemic nephrosis associated with massive hepatic

necrosis. Cortical ischemia and medullary hyperemia show a rather similar distribution and incidence. Figure 34 depicts the liver from the case of massive hepatic necrosis and figs 35, 36 and 37 show the kidney from the same case. The changes described in the distal portion of the nephron are apparent in these last three. Figures 39 and 41 illustrate other manifestations of the lesion in the proximal and distal tubules and their respective livers are illustrated in figs 38 and 40.

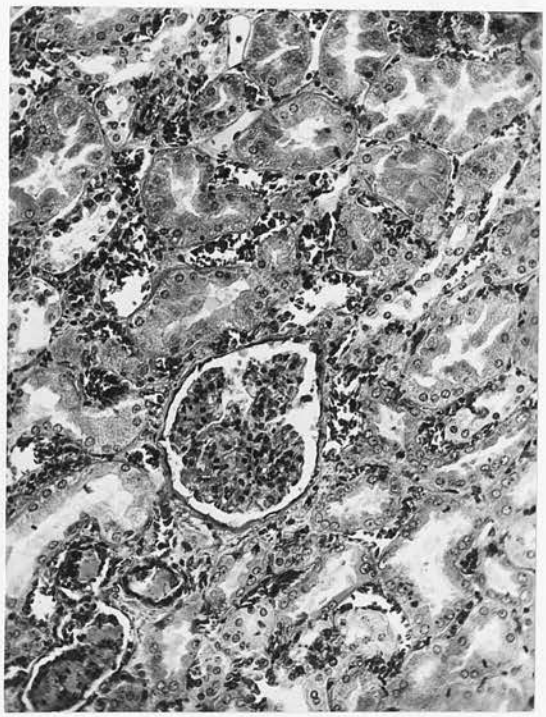


Fig.35. A-394, Case No.31. Trichrome, x150. Kidney from same case as in fig.34, dis-:playing the acute lesion in the proximal tubules of cytonuclear degeneration and sloughing, and the chronic atrophic changes of the epi-:thelium of the distal tubules in the region of 'foreign body' bile-pigmented casts.

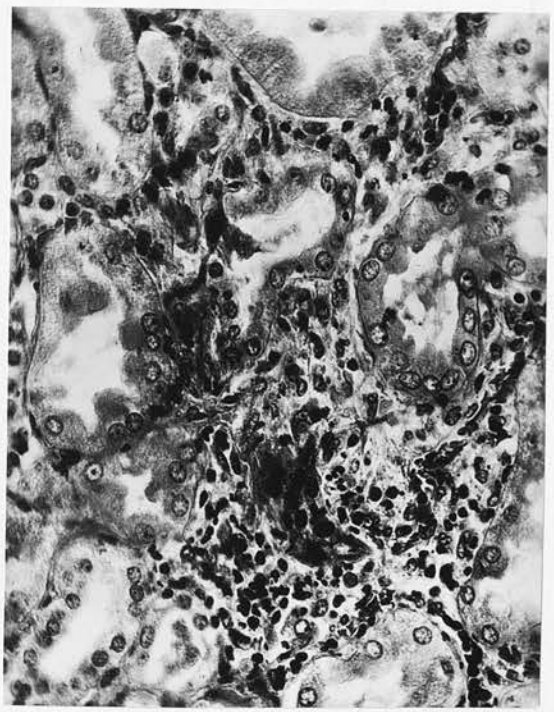


Fig.36. A-394, Case No.31. Trichrome, x300. Kidney from same case as in figs 34 & 35, showing the typical inter-:stitial changes of the so-called 'lower nephron nephrosis', usually interpreted as the result of tubal rupture and extrusion of protein, etc. casts.

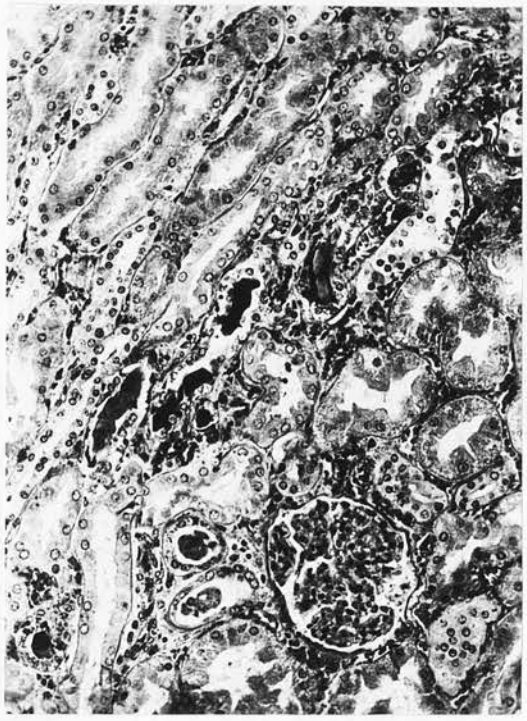


Fig.37. A-394, Case No.31. Trichrome, x150. Kidney from same case as in figs 34, 35 and 36, showing the acute cytonuclear changes and minor vacuolar degeneration of the proximal epithelium plus the atrophic degener-:ative changes surrounding bile-pigmented casts in the distal nephron.

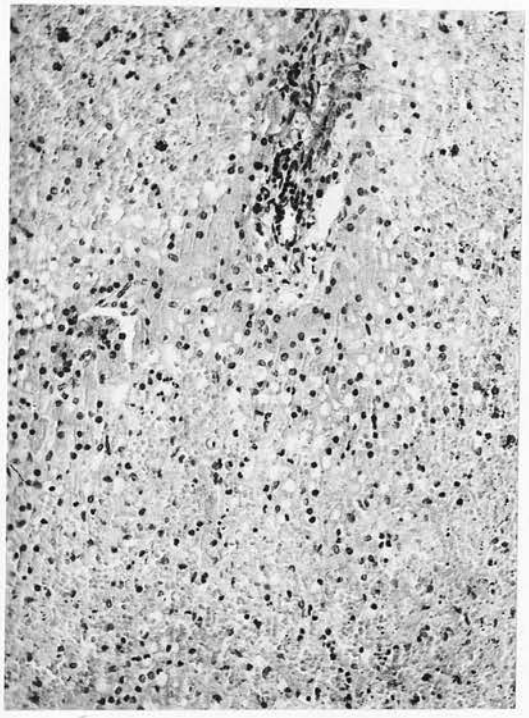


Fig.38. A-499, Case No.47. H&E, x150. Liver from case of confluent centrolobular necrosis of unknown (? toxic) etiology. Surviving liver tissue shows up as narrow bands in wide fields of hemorrhage and necrosis.

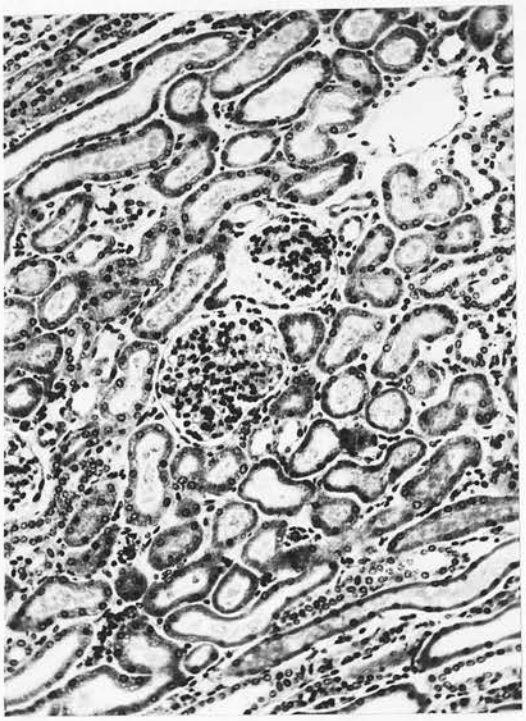


Fig.39. A-499, Case No.47. H&E, x150. Kidney from case illustrated in fig. 38. Note changes which are practically identical to those in fig.29. There is moderate swelling of the capsular epithelium.

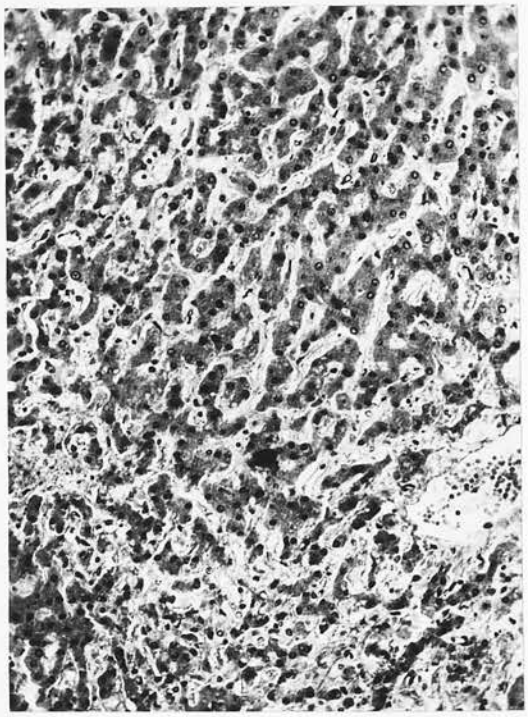


Fig.40. A-446, Case No.35. Trichrome, x150. Liver from a case of thymoma in which the tumor obliterated the chest cavity. Severe chronic venous congestion and incip-:ient fibrosis are seen.

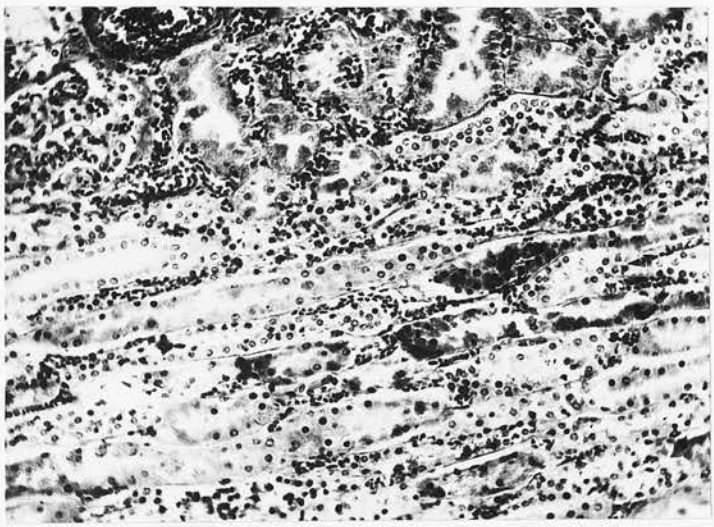


Fig.41. A-446, Case No.35. Trichrome, x150. Kidney from case illustrated in fig.40., showing the acute degenerative lesion in the glomerulus situated at the left-hand margin of the photograph and in the proximal and distal convoluted tubules.

Discussion

The significant fact established from this study is the constant association of a degenerative renal glomerular and tubular lesion with the presence of liver damage of varying type and degree. The renal lesion found in the selected group was similar in quality to the nephrotoxic and tubulorrhextic lesions that Oliver et al (1951) described so well in cases of acute renal failure. However, except for those cases of obvious severe renal damage diagnosed as 'lower nephron nephrosis' in the autopsy protocols, the changes were, by comparison, minimal. I have decided to call this lesion 'glomerulotubular nephrosis', but the terms 'nephrotoxic necrosis' and 'tubulorrhexis' of Oliver, 'glomerulonephrosis' of French (1950), or 'acute tubular necrosis' of Bull and Dible (1953), may be just as useful. It is obvious that no term can, as yet, be applied to these lesions that has either a pathogenetic or etiologic connotation. Since terms like 'lower nephron nephrosis' conjure up a picture of acute renal failure and are anatomically incorrect, it would be preferable to use another anatomical term to describe the renal lesion that appears to bear such a constant association to structural hepatic disorders.

The exact significance of the relationship between the hepatic and renal lesions cannot be discerned from the findings in this study alone. However, the facts suggest that morphologic damage with resultant dysfunction of the liver may be a preceding cause in the chain of events leading to the renal lesion. This possibility is supported by the constancy of the type of renal change and the wide variability of the inevitably associated structural changes in the liver. Assuming, then, that the damage to the liver precedes that to the kidney and is in any way responsible, it might yet be argued that the changes observed in many of the livers are too minimal to produce any recognizable hepatic dysfunction. On the other hand, Norcross et al (1951), in a study of 82 patients by a battery of liver function tests plus surgical biopsy of the liver, were unable to correlate morphological changes with abnormalities as detected by the function tests. They found the morphology to be little altered in the face of functional derangement. It is admittedly impossible to deduct on a vice versa basis from such findings, but it is tempting to suggest that if liver function can be impaired in the presence of normal histology, it is probably impaired when lesions are demonstrable, even if of varying type and minimal degree as found in most of our cases. In any case,

renal damage secondary to various forms of liver disease has been reported quite frequently in the medical literature, viz: Fahr described "bile nephrosis" in 1925; Furtwaengler reported the first case of hepato-renal syndrome in 1927; Heintzelmann (1947) and Farquhar (1949) have recorded abnormalities of renal function in cases of infectious hepatitis; Baxter and Ashworth (1946), Epstein et al (1950) and Patek et al (1951) have shown an interesting correlation between cirrhosis of the liver, impaired renal function and chronic "intercapillary" glomerulonephritis, while Morrison (1947) reported improved renal function in cases of nephrosis plus cirrhosis following dietary treatment of the cirrhosis; French (1950) noted that the state of the liver was related to "glomerulonephrosis".

Tomb in 1942, postulated an anoxic basis for the various clinical forms of acute renal failure and subsequently the ischemic etiology of the lesions of the anuric kidney has become increasingly widely accepted, (Oliver et al,(1951); Allen (1951); Sheehan and Moore (1952) and Bull and Dible (1953)). The features of glomerulotubular nephrosis certainly suggest focal ischemia as a potential factor in its patho-:genesis. When the correlated hepatic changes are considered, the interesting possibility arises that

circulating vasopressor substances, normally removed by the liver, tend to accumulate during a period of hepatic insufficiency and produce an acute ischemic state in the kidneys. Such a postulate might possibly shed some light on the curious and unexplained diuresis seen suddenly around the 10th to the 14th day in cases with acute renal failure, suggesting that the resurgence of renal function might depend upon the adequate resumption of the process of detoxification by the liver.

Despite its plausibility, it is impossible to prove the above hypothesis on the basis of the material in this study and a few alternative explanations should be mentioned.

1. Earlier investigators of the hepato-renal syndrome (Furtwaengler (1927), Helwig and Schutz (1932) and Pytel (1936)) favored the view that necrotic liver cells released a nephrotoxin into the circulation, or that alternatively, hepatic necrosis caused failure of detoxification of a toxin formed in the gut which thus passed directly into the systemic circulation. The former of these views is disproven by the absence of liver necrosis in 50% of cases in the present study; the latter view, while not disproved, is considered unlikely.

and enzymes (e.g. acetylcholine-esterase and the less specific adrenolytic "amino-oxidase"), and endogenous and exogenous (enteric) vasopressor materials.

Should the stated opinion regarding the hepatic causation of the renal lesion prove correct, it follows that I am, in effect, dealing with the earliest manifestations of something quite akin to the hepato-renal syndrome. Since this syndrome is already vague and somewhat ill-defined, it seems reasonable that its concept should be enlarged to include the findings of the present study, rather than to belabor the literature with unnecessary new terminology.

Summary

1. Glomerulotubular nephrosis, the basic histological pattern of acute degenerative lesions in the nephron, is described in detail.

2. A close correlation is found to exist between glomerulotubular nephrosis and structural changes in the liver.

3. The hepatic changes consist of acute and chronic lesions, and vary from severe fatty metamorphosis, cirrhosis and acute edema, through all stages of atrophy

and minor necrosis to massive hepatic necrosis.

4. The hypothesis is proposed that vasopressor materials accumulate in the systemic circulation during periods of hepatic insufficiency, and produce renal ischemia.

5. The correlation is felt to comprise part of the 'hepato-renal syndrome', and it is recommended that the syndrome, already nebulous and ill-defined, be extended to include these related phenomena.

SECTION III

SECTION III

Production of Acute Glomerulotubular Nephrosis in the Rabbit by means of Hepatic Surgery .

The previous sections have dealt with the natural incidence of hepatic lesions and co-existant glomerulotubular nephrosis (hereinafter referred to as G.T.N.) in human autopsy material and their differentiation from autolytic tissue changes. G.T.N. was shown to consist of focal degenerative changes in glomeruli and tubules. The present investigation was undertaken in an attempt to study the influence of acute experimental liver damage on the genesis of G.T.N. lesions in the kidney of the rabbit.

Several earlier attempts are recorded in the literature. Helwig and Schutz produced hepatic and renal necrosis in dogs by occlusion of the hepatic artery and by traumatic rupture of the liver in 1932. In 1935, Boyce and McFetridge were unsuccessful in producing renal lesions in rabbits by similar procedures, though they succeeded in doing so through the relief of temporary (2 week) biliary obstruction. The following year, Pytel (1936) using the techniques of Helwig and Schutz (loc.cit) succeeded in the experimental production of the hepato-renal syndrome in rabbits. In view of these conflicting reports it

was decided to approach the investigation along slightly different lines, avoiding, where possible, such complications as shock, cholemia and chronicity. The object of the experiments was to produce a state of acute, uncomplicated, hepatic damage in rabbits and to assess the influence of such a condition upon the otherwise normal kidneys.

Materials and Methods.

Forty nine young (1300 to 2500 gram) rabbits of both sexes were utilized. An exploratory pilot experiment was devised to determine the most satisfactory approach, using 11 rabbits. The operative procedures employed included ligation of either the porta hepatis, the portal vein, or the hepatic artery in the free zone of the porta (total of 5 rabbits) and temporary occlusion of the porta hepatis (6 rabbits). After this preliminary study, further investigation was conducted along the following lines: (a) temporary occlusion of the porta hepatis in 12 rabbits. Porta clamped off for periods up to 30 minutes as conditioned by the clinical status, (b) unoperated controls (17 rabbits), (c) simple laparotomy controls (9 rabbits).

All operations were carried out under open-ether anesthesia.

Through a 3 inch median incision the porta hepatis was cleared as gently as possible and the clamp applied. The latter consisted of Willets forceps whose blades were encased in firm gum rubber tubing so that the rubber surfaces were in gentle apposition. The clamp was allowed to rest against the rib margin and the incision was sutured with cotton in two layers from the lower end of the incision to within one inch of the portal clamp. Ether was given as required and the clamp was left in position for as long an interval as possible up to a maximum of 30 minutes. (Usually between 15 and 20 minutes, to avoid shock). The animals were usually conscious within a few minutes following cessation of the operation. In the two laparotomy control animals of group F, the left renal pedicles were occluded for 10 minute periods. Post-operatively all animals were placed in metabolism cages and the urine output recorded. Urine for urinalysis was obtained at time of autopsy as a bladder specimen by means of a syringe and needle. Animals which died during the operation were autopsied immediately. The remainder were killed by air-embolism approximately 24 hrs post-operatively.

One animal, A-6, died 8 hrs post-operative and three were killed later than the 2nd post-operative day (99, A-7 and A-3; forty eight, forty eight and seventy two hours respectively).

Portions of the right and left hepatic lobes and one half of each kidney were fixed in Zenker-Formol (80% Zenker's solution and 20%neutral 100% formalin) for 8 to 12 hours and washed in running water for 24 hours. All blocks were then treated in identical fashion, being dehydrated, cleared and embedded in paraffin. Sections were cut at 5 microns and stained with H&E and Masson's Trichrome in all instances, while in 10 cases, frozen sections of formalin-fixed tissues were stained for fat with Sudan IV.

Results.

Clinical. Ten animals died either during the operation or in the immediate post-operative period, a mortality of approximately 40%. Survivors are observed to enter a phase of acute oliguria in which the 24 hr urine volume drops to about ¼ of the normal as recorded in control laparotomy animals. Anuria is never encountered in these short-term experiments.

Characteristic post-occlusive urinary findings include albumen, trace to +; bile ±; hyaline and granular casts, 0 to 1 / H.P.F.; R.B.C's 0 to 2 / H.P.F., uncentrifuged. Pre-operative urinalyses are negative in the few cases that were so investigated. The animals which survive appear in good condition apart from the one which died 8 hrs post-operatively. They are noted to take both food and drink the day of the operation and to show no untoward listlessness in excess of that shown by the laparotomy controls.

Morphological. The macroscopic features at autopsy vary somewhat according to the procedure employed during the operation. In the group in which the porta hepatis was clamped, the livers reveal some increase in the lobular markings plus a moderate degree of pallor which imparts a mottled tawny appearance, in striking contrast to the normal reddish-brown livers of the control animals. Where ligation of either the portal vein or the hepatic artery was performed, massive, grey-green necrosis and peritonitis is found in varying stages of development, limited largely to the right lobe(s) and spreading to the left to produce focal involvement of the median lobe. In addition to induced hepatic disorders there is found a surprisingly high incidence (43%) of naturally occurring diseases in the

livers, of which coccidiodal infestation comprises more than half (27%). The kidneys show no constant macroscopic changes, some appearing slightly hyperemic, others displaying a slight pallor. Usually they are normal in appearance.

Microscopically the hepatic lesions vary from focal through confluent to include massive necrosis, the area of involvement corresponding to the macroscopic picture. In addition, the coccidiodal foci and various lesser 'natural' lesions (focal and centrolobular necrosis, serous and chronic periportal hepatitis and cirrhosis) complicate the picture, when present.

Figures 42 to45 inclusive depict the commonest of the hepatic lesions.

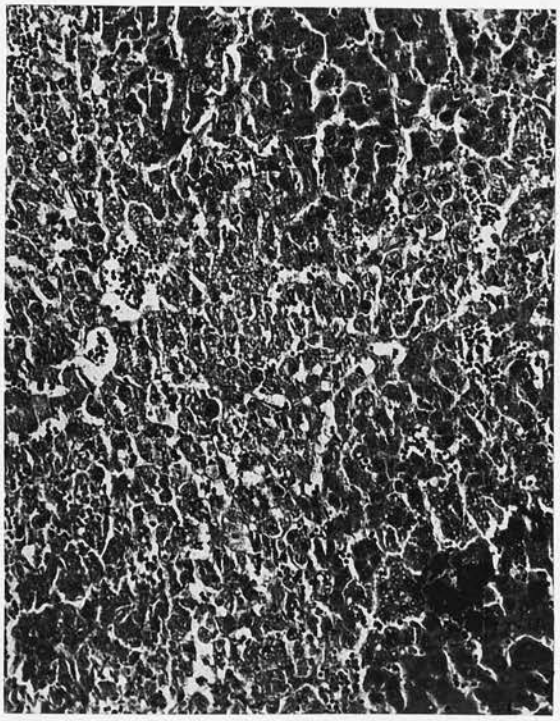


Fig.42. No.A.7. Rabbit liver. Trichrome, x150.Extensive necrosis developing after li-:gation of the hepatic artery above the origin of the gastro-duodenal artery. Animal killed 48 hrs later.

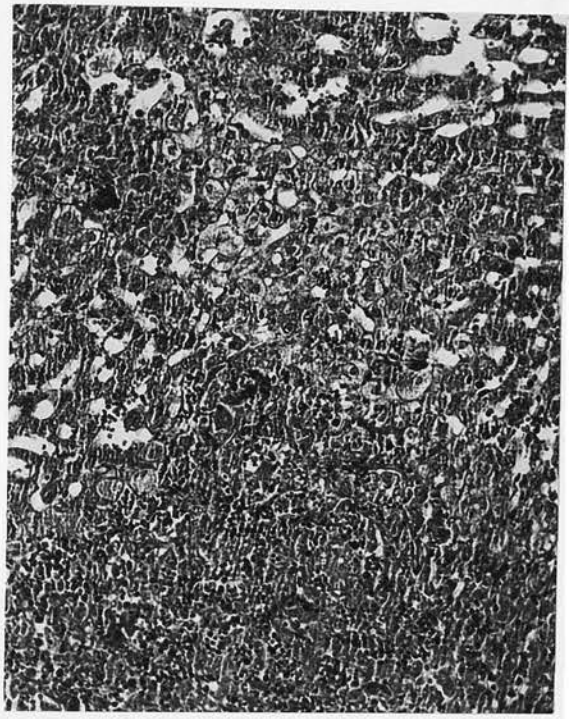


Fig.43. No.99. Rabbit liver. Trichrome, x150. Extensive necrosis following application of clamp to the porta hepatis for a 30 minute period. Animal killed after 48 hrs.

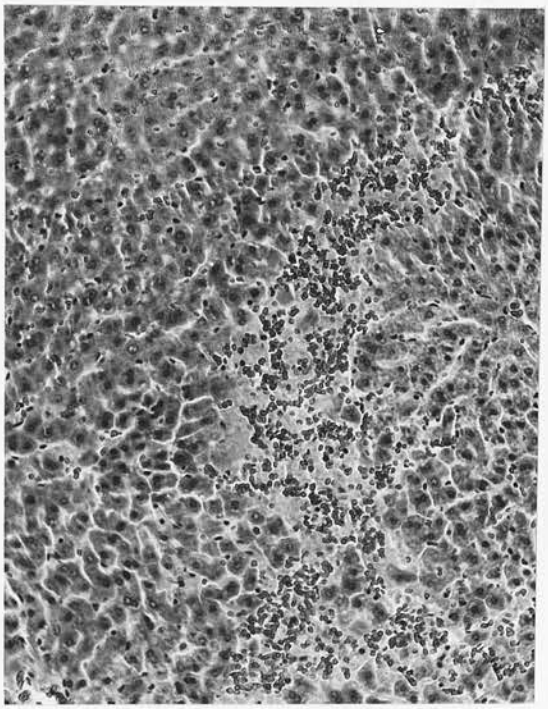


Fig.44. No.A.5. Rabbit liver. H&E x150. Confluent hemorrhagic necrosis (minimal) 24 hrs after a 10 minute application of clamp to the porta hepatis.

Fig.45. No.B.13. Rabbit liver. Trichrome, x40. Three foci of coccidiodal mycosis with widespread intervening necrosis of parenchymal tissue. Surviving liver tissue represented by dark bands in the photograph.

The renal lesions correspond to those seen in human G.T.N. (Section II). As emphasized in the latter report, the lesion in acute G.T.N. is best visualized through use of the Trichrome stain. The selectivity of this stain is well seen by comparison of figs. 46 and 47.

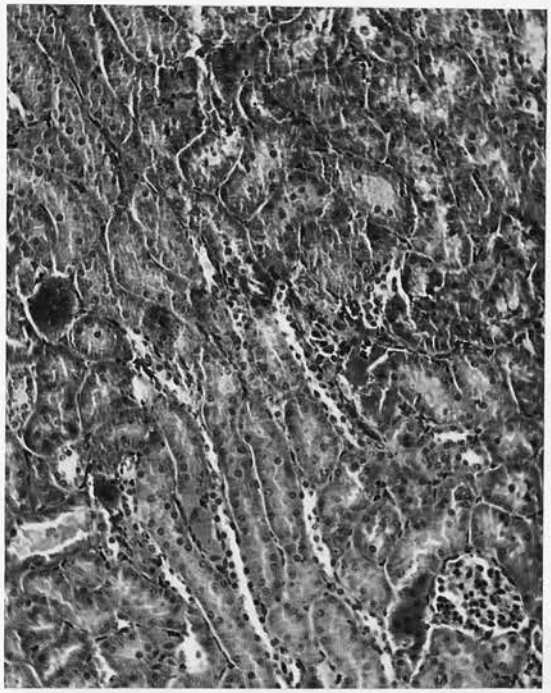


Fig.46. No.B.15. Rabbit kidney. H&E x150. Patchy acute G.T.N. in the proximal convoluted tubules as evi-:denced by nuclear pyknosis and dense eosinophilia of the cyto-:plasms. Note the presence of protein-containing fluid throughout the nephron.

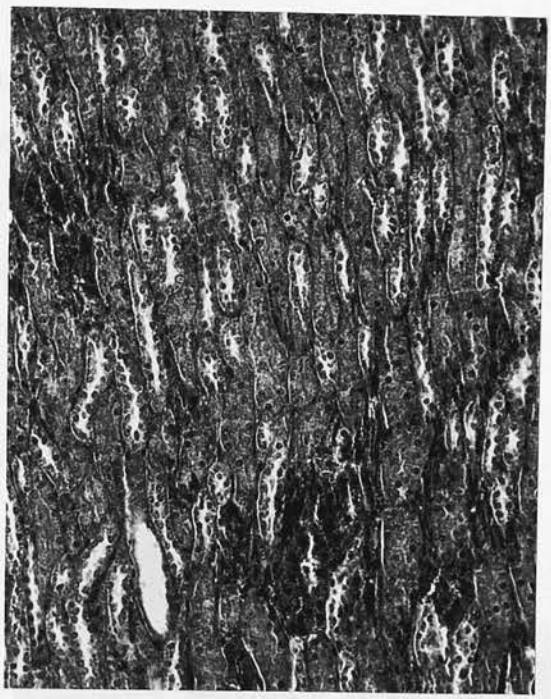


Fig.47. No.B.15. Rabbit kidney. Trichrome, x150. Section from an area in close proximity to that shown in fig.46, displaying acute G.T.N. in the distal portions of the proximal tubules.The changes show sharper contrast with surrounding normal tissue than is seen in fig.46.

In acute G.T.N. the glomerular capsular spaces are seen to contain proteinous fluid. There is minimal to slight swelling and desquamation of the capsular epithelium. In both the proximal and distal convoluted tubules there are noted hyaline or colloid casts, plus a patchy, bright red, smudgy or finely granular degener-:ation of the cytoplasm and pyknosis of the nucleus so characteristic of acute degeneration or necrosis of the epithelium viewed in trichrome-stained sections. These

epithelial changes are focal, affecting odd cells in the majority of nephrons but tending to occur in patchy groups of tubules in the cortico-medullary segments of the proximal tubules (Fig.47). Red blood cell casts at various levels of the nephron are a not uncommon finding. Several features of the acute lesion are depicted in figs. 48 and 49, in addition to those seen in figs. 46 and 47.

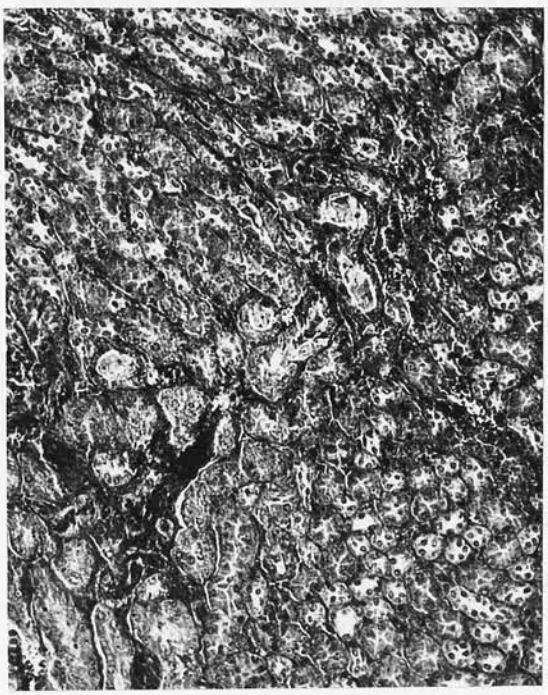


Fig.48. No.A.43. Rabbit kidney. Trichrome, x150. Acute G.T.N. depicting degenerative epithelial changes in the cortico-medullary segments of the proximal tubules plus proteinous fluid in tubal lumina and several casts composed of sloughed epithelium.

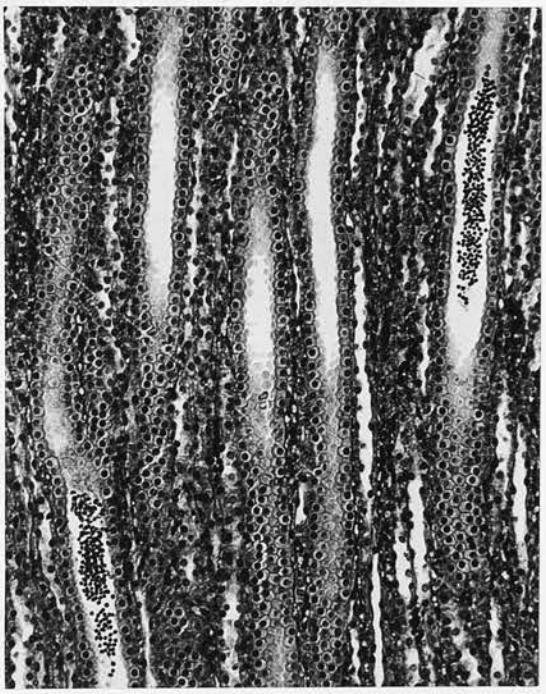


Fig.49. No.A.33. Rabbit kidney. Trichrome, x150. R.B.C. casts in the lumina of collecting tubules in a case of acute G.T.N.

With the aid of high magnification, the cytoplasmic changes are shown to represent a disruption of the normal rod shape and polarity of the mitochondrial elements. These bodies normally stain bright red with ponceau-fuchsin, but, containing the dye within their fine fascicles, they have no profound effect on the staining intensity of the cytoplasm. When the cell is damaged the rodlets either disrupt entirely, smearing the red-staining material throughout the cytoplasm (fig.50), or they fragment and agglomerate with the formation of tiny beads. These beads appear identical to those seen in mercurial renal disease as shown by Ogilvie in 1932. The changes do not occur in post-mortem autolysis. In addition to the finding of degenerate and necrotic cells 'in-situ', sloughed individual cells or cell clumps are easily demonstrated in the lumina at various levels of the nephron.

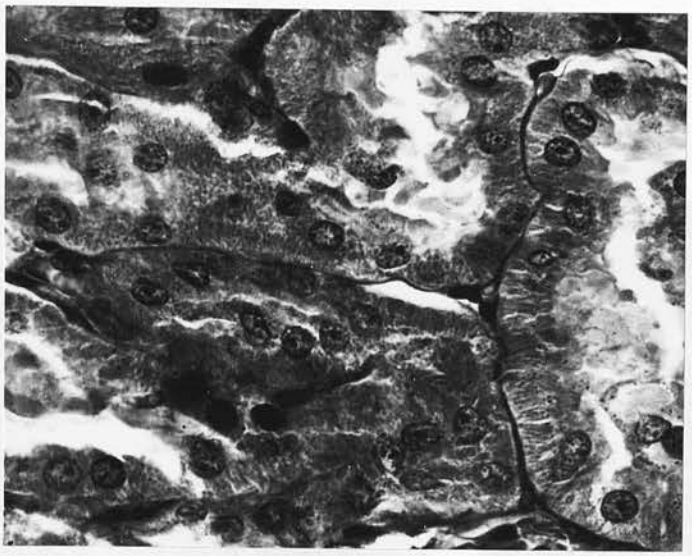


Fig.50. No.A.8. Rabbit kidney. Trichrome, x750. Acute G.T.N. The group of three contiguous pyknotic nuclei mark acutely degenerated cells in which the mitochondria have disappeared and their substance is smeared throughout the cytoplasm.

In morphological sub-acute G.T.N. the lesions of the acute phase are often intensified. There is more pronounced swelling and sloughing of the capsular epithelium in the damaged glomeruli and formed casts of tubular epithelium appear in the distal and collecting tubules, amounting, in some instances, to the typical "renal failure casts" of Addis. In the later stages of the sub-acute process some degree of epithelial proliferation in the glomerular tuft and Bowman's capsule; hyaline, granular and cellular casts in distal and collecting tubules and early interstitial granulomatous and round cell infiltrations are noted.

Most of the above features are well portrayed in figs. 51 and 52.

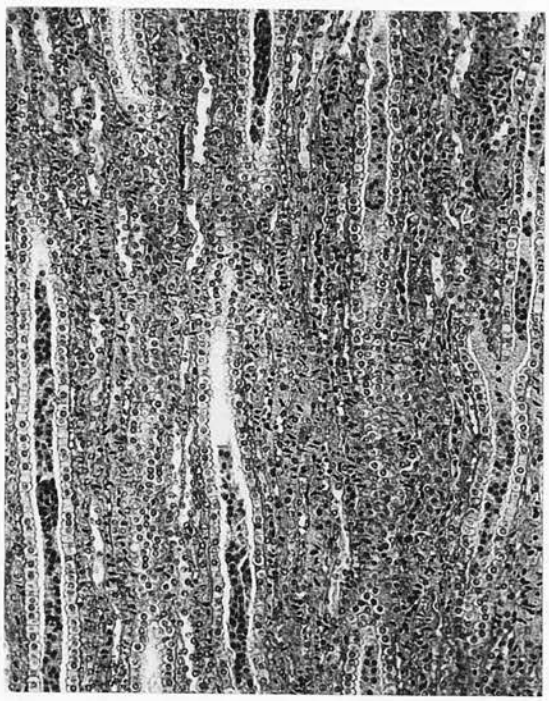


Fig.51. No.A.41. Rabbit kidney. Trichrome, x150. Sub-:acute G.T.N. Large mixed cellular and granular casts are seen plugging the collect-:ing tubules. That they are of fairly recent origin is borne out by the good state of preservation of the compo-:nent cells.

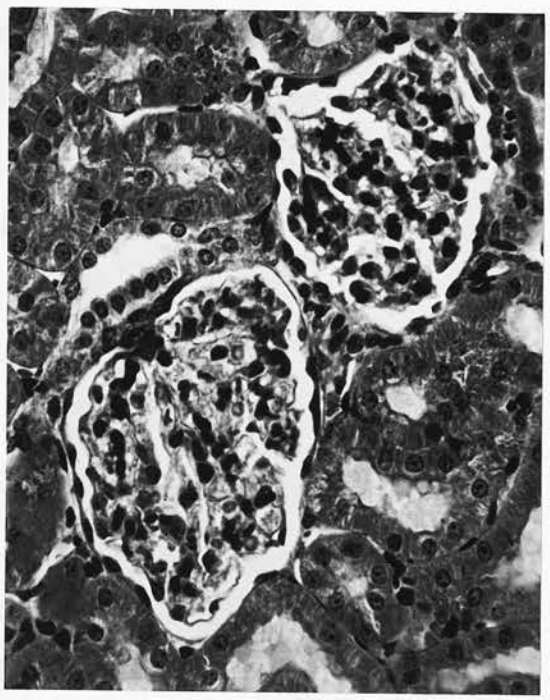


Fig.52. No.B.24. Rabbit kidney. Trichrome, x400. Subacute G.T.N. Proliferation of the epithelium of the tuft and capsule, with synechiae is seen in the smaller glomeru-:lus. Several tubules contain proteinous fluid.

Correlation of Renal and Hepatic lesions. Owing to the high incidence of native hepatic disease and G.T.N. in the animals comprising the nucleus of the present investigation it has been necessary to re-organize the groups for the presentation of results. Table 4(appendix)

displays the incidence of G.T.N., correlating it with "medical" hepatic disorders in Groups A & B, and with experimental surgical hepatic lesions in Group C. Group B is composed of animals in which a surgical procedure accompanied the medical lesion in the liver. Group D consists of the normal controls while Group E contains both simple laparotomy controls and animals which underwent surgery but died under the anesthetic. Group F consists of two animals in which temporary (10 minute) occlusion of the left renal pedicle was performed during the course of a 20 minute laparotomy. The table records the surgical procedure, if any; the presence or absence of complicating hepatic disease; the weight of the animal; its clinical course; the histopathology of the liver; the post-operative urin-:alysis, where applicable; and the degree of G.T.N. In the recording of the latter, - indicates the absence of any form of G.T.N.; + and ++ indicate the acute phase of G.T.N. graded according to numerical distri-:bution among the nephrons; and +++ and ++++ indicate subacute G.T.N. graded in like manner. Table 5 briefly summarizes the incidence of the hepatic and renal lesions recorded in Table 4 (appendix).

TABLE 5

Summary of Incidence of Hepatic and Renal Lesions.

Rabbits with liver damage	No.	No. with G.T.N.
Group A - medical diseases of liver	8	7
Group B - medical & surgical diseases of liver	13	13
Group C - surgical diseases of liver	10	9
Total	31	29
Rabbits without liver damage		
Group D - normal controls	9	1
Group E - laparotomy controls & operative deaths	7	0
Group F - laparotomy & occlusion of left renal artery	2	(0. R.kidney (2. L.kidney
Total	18	1

Of the 31 animals with liver damage listed in Groups A, B and C, Table 5, 29 display varying degrees of G.TN. Of the two which failed to show renal damage, one reveals slight coccidiodosis of the liver and the other has undergone clamping of the porta hepatis for a 10 minute period. (No liver lesion developed in the latter instance). Post-operative

urinalysis reveals renal damage in 12 of the 13 cases so investigated. Their pre-operative urine specimens were normal. In the purely surgical group (C), the hepatic lesion is invariably some form of necrosis. Though usually focal and patchy following clamping of the porta hepatis, several exceptions with more severe lesions such as centrolobular, confluent and massive necrosis are present. Where ligation of one or other of the vascular components of the porta was performed, massive necrosis is the inevitable sequela.

17 of the 18 animals in Groups D, E and F have no lesions of the liver or kidneys apart from the unilateral acute G.T.N. occurring in the 2 cases in Group F after clamping off the left renal pedicle for a 10 minute period. The remaining animal does show the lesions of acute G.T.N. in the absence of provable hepatic damage. The liver in this instance contains tiny foci of acute degeneration, marked by nuclear pyknosis and cytoplasmic polychromasia, but definite necrosis is lacking.

Thus it is found that G.T.N. correlates with disease of the liver in roughly 94% of experimental animals, of which approximately 63% of the liver disease is of non-surgical etiology. In this study it occurs in otherwise normal rabbit kidneys without proven

hepatic disease in approximately 2% of cases.

In order to establish the identity of the histo-pathology of acute G.T.N. with that seen in true renal ischemia, the left renal pedicles were clamped off in the 2 rabbits of Group F for 10 minute periods. The animals were otherwise treated as simple laparotomy controls as outlined previously. Only one animal developed abnormal urinary constituents, but both show a well developed focal renal lesion, indistinguishable from acute G.T.N., in sections from the left kidneys (fig. 53). The right kidneys are normal.

Variable results are observed in the 10 cases stained for fat, both in the kidney and the liver. It would appear that fatty degeneration is not a feature of the acute or subacute lesion of G.T.N. and that the fat content of the liver is independent of all of the disease processes encountered in this series.

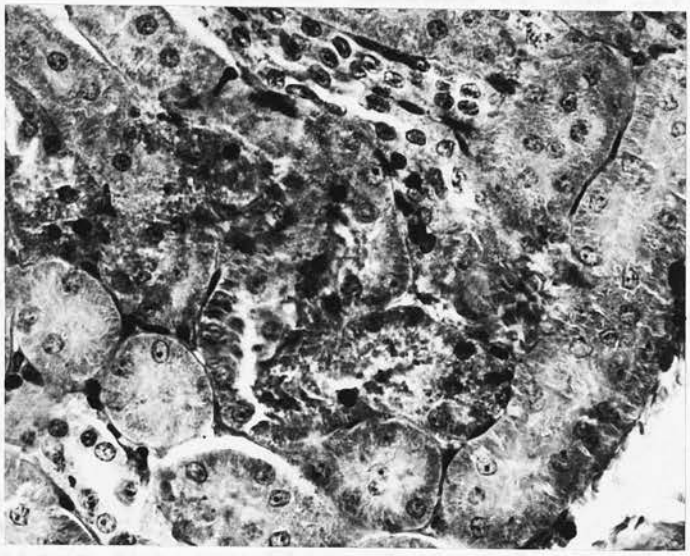


Fig.53. No.A.31. Rabbit kidney, Trichrome, x400. Anoxic nephrosis. The tubular lesion is identical with that of acute G.T.N. as shown in figs. 46, 47, 48 and 50.

Discussion.

This investigation establishes an impressive correlation between the presence of liver damage and a renal lesion designated as glomerulotubular nephrosis. A similar correlation in human autopsy material was noted in Section 2 of this text. Because of this latter observation, the present experiments were first designed to study the effect of experimental acute uncomplicated liver damage on structure and function of the kidney. However, the high incidence of natural disease in the liver made it impossible to analyse the effects of uncomplicated acute liver damage on the kidneys and on final evaluation of this experiment it

was clear that natural disease of the liver, alone or in combination with simple laparotomy or terminal acute surgical hepatic damage, as well as acute surgical damage of the liver was correlated with renal G.T.N. This was in contrast to the absence of renal G.T.N. lesions in animals with no natural or experimental liver disease. The correlation of natural disease of the liver and G.T.N. lesions heightens the importance of the association of liver damage and renal G.T.N. and raises the question of the advisability of the use of rabbits for the experimental investigation of renal and/or hepatic diseases.

Extreme liver dysfunction with acute renal failure has been recognized for some time under the term hepato-renal syndrome. Renal lesions of this entity possess the basic features seen in so-called 'lower nephron nephrosis'. In the preceding Section and in this detailing the morphology of G.T.N. lesions it has been pointed out that the elementary changes are qualitatively identical to those described by Oliver (1951) in dissected nephrons from cases of acute renal failure. Many investigators have attempted to duplicate experimentally the clinical and pathological features of the hepato-renal syndrome (Helwig and Schutz, 1932; Boyce and McFetridge, 1935; Pytel, 1936, and others).

In most of their experiments the liver lesions were usually chronic and have been complicated by cholemia and shock. The present study was planned to investigate the effect of uncomplicated liver damage on the kidney without producing such profound changes as seen in the hepato-renal syndrome. In this way it was hoped to isolate more exactly the role of the liver in damage to the kidney than could be determined from natural human disease or experiments where more profound metabolic changes such as shock and cholemia are occurring. The findings of the present experiments suggest that minimal disease and dysfunction of the liver produces a microcosm of the hepato-renal syndrome and indicates that liver damage may be an essential factor in the genesis of the G.T.N. lesion which is basically similar to the renal lesion of the hepato-renal syndrome. These findings may indicate that liver function influences kidney function and structure in some general way not as yet understood.

In the present study, it would be interesting to know the ultimate mechanisms responsible for the renal lesion and the relationship of liver damage to these mechanisms. That the renal lesions could be due to renal ischemia seems reasonable. They have the essential elements of 'lower nephron nephrosis' in which there is good evidence for ischemic pathogenesis,

as noted by Tomb (1942), Oliver et al (1951), Sheehan and Moore (1952) and Bull and Dible (1953).Furthermore, in two of my rabbits, temporary clamping of the renal pedicle produced a similar lesion. On the other hand in the present study, while it seems reasonable that renal vasospasm might accompany acute surgical damage of the liver, credulity is strained to suppose renal vasospasm accompanies the chronic natural disease of the liver. In any case, to prove that the lesion of glomerulo-:tubular nephrosis is of ischemic genesis and a forerunner of 'lower nephron nephrosis', it would be necessary to make serial time studies on the development of experi-:mental 'lower nephron nephrosis' with its accompanying renal vasospasm and determine if the early lesions were identical to G.T.N. Lesions which vary in intensity from albuminuria and casts in the collecting tubules, through acute tubular necrosis to focal and generalized renal cortical necrosis have been reported by Sheehan and Moore (1952), in eclampsia and utero-placental apoplexy in humans and by Block et al (1952, a & b) in exsanguination studies in the dog and rat. The mild and intermediate forms of these lesions would appear to be identical to acute G.T.N. In both instances the authors attribute the etiology of the renal lesions to vasospasm.

Whatever the ultimate mechanism of the G.T.N. lesion, it is important to assess the role of the liver in the overall chain of events. In severe liver disease with the development of the hepato-renal syndrome and in severe experimental liver damage it has been suggested by Furtwaengler (quoted in Pytel, 1936) that renal vasospasm is mediated through hepatic dysfunction by the accumulation of a nephrotoxic material in the blood whose effect is produced on the walls of the blood vessels. In the previous Section I have likewise stressed the plausibility of such a pathogenetic mechanism. However, in the present investigation there is no proof that the damage to the liver results in abnormal vaso-:spastic materials in the circulation, nor can temporary renal vasospasm secondary to acute temporary shock accompanying the surgical damage of the liver be ruled out, though the occurrence of G.T.N. without shock in natural liver disease tends to minimize the importance of the latter.

To establish that the genesis of the renal damage is secondary to liver damage by way of either the accumulation of renotoxic or renal vasopressor substances requires further experimentation. The salient factors which have emerged from these studies are the close correlations between renal G.T.N. and natural

hepatic disease on the one hand (95%) and all forms of hepatic damage, both natural and experimental, on the other (94%). Even more unusual is the fact that the natural diseases were, on the whole, minimal in degree and could not be expected to produce severe, generalized, non-specific disturbances of homeostasis. Such a finding suggests that there is something unique about damage to the liver as contrasted with damage to other tissues.

Throughout the present and the earlier investi-:gations the important contribution of the Masson Trichrome stain has been stressed. Through this medium, renal tissue which may at first sight appear normal in H&E sections will often show acute damage, changes which can subsequently be traced out in H&E sections. The stain requires considerable technical skill, the chief source of error lying in the differ-:entiation with picric-alcohol. This step is not complete until all hematoxylin has been removed from the cytoplasm of the epithelial cells of the tubules, as its presence in this site tends to mask the subsequent uptake of red dye from the ponceau-fuchsin stain. (I have found the most satisfactory ponceau dye to be "Ponceau 2 R", color index 79, by Harleco.) The

timing of this step is aided by the simultaneous disappearance of hematoxylin from the cytoplasm of glomerular tufts and its practical disappearance from tubular epithelial nuclei. It is not improbable that, using this stain procedure, lesions which have been recorded as " + G.T.N." are considerably less advanced than those usually reported in the literature in entities such as acute tubular necrosis, etc.

Summary

1. An attempt has been made to experimentally reproduce the correlated hepatic and renal lesions reported in human autopsy material in Section II by means of temporary occlusion of the hepatic vasculature in the rabbit. The object of this procedure was to produce a state of acute hepatic insufficiency without jaundice. Glomerulotubular nephrosis was found to develop in 90% of these animals.

2. A rather large number of the rabbits used in this investigation (43%) showed pre-existing hepatic lesions of varying types and duration. These hepatic changes showed a 95% correlation with degenerative renal changes, causing major re-organization of the

experimental groupings. The rabbit is thus felt to be unsuitable for studies of experimental hepatic and/or renal diseases.

3. A lesion identical to acute glomerulotubular nephrosis has been produced in two rabbits by direct renal ischemia.

4. The proposed ischemic etiology of G.T.N. has been discussed in the light of the associated hepatic disorders, a possible sequence of events being as follows: hepatic insufficiency develops in the presence of a variety of lesions; the insufficient liver fails to detoxify some potent vasospastic material in the circulation; this agent produces selective arteriospasm in small renal vessels.

5. The Masson Trichrome stain is recommended for the investigation of acute degenerative lesions of the kidney and a note is included on some of its more important technical features.

SECTION IV

SECTION IV

Studies on glomerulotubular nephrosis as induced by hepato- and nephro-toxic chemicals in the rat.

Preliminary investigations in this series have shown a striking correlation between the non-specific renal lesion of glomerulotubular nephrosis (G.T.N.) and various types of liver damage, both in the human (Section II) and in the experimental rabbit (Section III). As emphasized in the latter report, the possibility that the renal lesion might result from surgical shock could not be excluded. Indeed, it is theoretically impossible to induce acute hepatic ischemia in an otherwise normal animal by interference with the vascular supply of the liver without producing some alterations in the hemodynamics of other body organs.

The present investigations were instituted to observe the nature of chemically-induced hepatic and renal tubular necrosis, both in the acute and sub-acute forms and to assess the influence of a hormonal vaso-active substance (posterior pituitary extract) on the kidneys of rats with concurrent liver damage. By such procedures shock is circumvented except where large doses of corrosive sublimate are employed. On the other hand, an additional complication is introduced.

It is axiomatic to the thesis of the hepatic pathogenetic role in the renal lesions of G.T.N. that a selective hepato-toxin which noticeably impairs hepatic function cannot exist. The kidneys must automatically become affected. Nowhere is this more apparent than in chlorinated hydrocarbon intoxic-:ation, of which Allen (1951) classes the renal lesion as a form of cholemic nephrosis. This factor of dual toxicity can be minimized only by adequate control material and cautious interpretation.

The vast majority of animals in this present investigation were subjected to hepatic necrosis through the agency of carbon tetrachloride, with ethyl alcohol as an adjuvant drug to heighten the initial effect (as shown by Smillie and Pessoa, 1926) and to inhibit and prolong hepatic regeneration. A small number were treated with mercuric chloride, the primary interest here being the observation of associated vascular changes by arteriographic methods.

Materials and Methods.

Albino rats of the Glaxo-Wistar strain were employed throughout these studies. The animals were of mixed sexes and ranged in weight from 70 to 250 grams,

the mode being 110 grams. They were obtained from the standard breeding stock and fed the standard rat cube diet (Rat Cake Nuts, North Eastern Agric. Co-op. Society Ltd, Aberdeen) without supplement. Animals selected for experimentation (experimental and control alike) were segregated three days prior to use and given a diet composed of 1 rat cube per rat per day plus 6 % alcohol in their drinking water. The water also contained 2 ccs of vitamin B complex (Betalin, Lilly) and 0.625 grams of phenylindanedione anti-:coagulant per litre. Injected drugs were administered into the thigh muscles under light ether anesthesia. Carbon tetrachloride was administered by inhalation to the stage of unconsciousness in a large glass jar.

At selected intervals following the administration of the drug under investigation, variable numbers of rats, both experimental and control, underwent the following operative procedure. The first rat was given 0.1 cc of 10% W/V hexamethonium bromide by intramuscular injection and its weight and sex recorded. A ten minute interval was then allowed for the ganglion block to take effect. By this time the rat usually appeared huddled-up and relatively inactive and described vertigin-:ous movements when handled. Coarse respiratory rales

could be heard and felt and the respirations appeared somewhat laboured. The procedure was repeated with the next rat and the first one was placed under deep ether anesthesia.

The animal was then opened by ventral incision from pubis to xiphisternum and the incision was extended laterally to the mid-axillary line along the costal margins. The thoracic cage was opened at the lateral borders of the incisions, starting on the left side and the left leaf of the diaphragm was removed anteriorly to expose the heart. Into the vigorously beating left ventricle, 0.2 ccs of heparin containing 5000 I.U./cc were injected. The thoracic resection was then continued to the manubrium sterni bilaterally and the anterior half of the thoracic cage was removed. The left lung, the heart, the stomach and intestines and the liver were then shifted to the right sufficiently to adequately expose the thoracic aorta. The aorta was freed posteriorly for a distance of $\frac{1}{2}$ inch above the diaphragm and a cotton thread positioned beneath it at this point. The aorta was then nicked with fine, needle-point scissors and the polythene injection cannula introduced and ligated into position with the tip well proximal to the origin

of the renal arteries. Flushing with heparinized N-saline (25,000 I.U. heparin/litre) was commenced immediately and the aorta was occluded with artery forceps proximal to the bifurcation. A nick was made in the left renal vein and flushing continued until the kidney appeared bloodless (usually 30-40 seconds). The saline was then discontinued and the bismuth injection compound (30% bismuth oxychloride suspension in 10% plasma protein solution) was run in. After flow had practically ceased (usually 2-3 minutes) the kidneys and liver were then ligated at their hila and excised. The right kidney was hemisectioned longitudinally and, along with a portion of the right hepatic lobe, was placed immediately into Helly's fluid. The left kidney and remainder of the liver were either placed into 10% formalin or frozen with CO_2 snow for subsequent arteriographic studies as detailed in Section V. All formalin-fixed kidneys were weighed and the weights recorded.

The Helly-fixed tissues were washed in running water the following day for 24 hours. All blocks were then treated in identical fashion, being dehydrated, cleared and embedded in paraffin. Sections were cut at 5 microns and stained with hematoxylin and eosin and Masson's trichrome in all instances.

Preliminary Experiments.

A series of pilot experiments were designed to ascertain the histological response of the normal rat to the following drugs: (a) carbon tetrachloride; (b) ethyl alcohol plus phenylindanedione; (c) carbon tetrachloride and ethyl alcohol-phenylindanedione in combination; (d) special vasodilator drugs employed in the late pre-agonal period; and (e) intravascular saline flush followed by bismuth oxychloride.

(a) Carbon tetrachloride toxicity.

Procedure. Thirty of thirty-two rats were given carbon tetrachloride and then retained on routine animal care and feeding. From this group the two controls and two of the experimental rats were autopsied within 4 hours and on each succeeding day two further rats were autopsied, the observations thus extending over a two-week interval.

Results. In the livers, centrolobular vacuolar degeneration plus necrosis of parenchymal cells appears on the day following the intoxication (day 1) and persists with concomitant leukocytic infiltration until day 3. From day 4 to day 7 inclusive, evidence of previous damage and repair in the form of alterations of parenchymal cell staining qualities, small foci of cellular detritus and mitotic and multinucleate cord

cells persist. From day 8 onward, the livers appear histologically normal. In the kidneys, rat G.T.N. (to be described in detail under experiment 1) develops on day 1 and persists until day 3, being of maximum severity on day 2. An equivocal G.T.N. lesion is present in the kidneys of one of the two rats employed for day 4 and all subsequent kidneys are normal.

(b) Ethyl alcohol toxicity.

Procedure. Sixteen rats were placed on a diet containing 1 cube / rat / day plus 6% alcohol-phenylindanedione-betalin solution ad lib. Two rats were autopsied each day commencing the following day, covering an 8 day interval.

Results. No histological changes in the kidneys are observed throughout the series. The livers develop minimal, focal, fatty metamorphosis from the fourth day onwards.

(c) Combined alcohol-carbon tetrachloride toxicity.

Procedure. Twenty one rats were administered carbon tetrachloride and then fed 1 cube / rat / day plus the alcohol-phenylindanedione-betalin mixture. Three animals were sacrificed each day, starting the

day after the carbon tetrachloride intoxication, the investigations thus covering a one-week interval.

Results. The findings are identical with those of pilot experiment (a) except that hepatic and renal lesions are very slightly accentuated. The combined intoxication does not alter the day of appearance or disappearance of the lesions.

(d) Vasodilator-drug toxicities.(Pre-agonal administration).

Procedure. Twelve rats were placed on the alcohol-anticoagulant regimen for three days. One pair was retained as control and the remaining 5 pairs were given intramuscular injections of the following chemi-:cals 20 minutes prior to autopsy: Apresoline, 2 mgs, (Ciba, 1 hydrazinophthalazine hydrochloride); Vegolysen, 10 mgs, (May & Baker, hexamethonium bromide); Priscol, 2.5 mgs, (Ciba, 2-benzyl-imidazoline-hydro-:chloride); Pronestyl, 10 mgs, (Squibb, procaine amide); and Rogitine, 1 mg, (Ciba, 2-[N,p-tolyl-N(m'hydroxyphenyl)-amino-methyl] imidazoline).

Results. No histological alterations of note can be ascribed to the use of these drugs in either kidney or liver. The organs appear somewhat more congested

than normal upon macroscopic examination in-situ, but this appearance, along with any accompanying histological change, is lost when they are flushed with saline.

(e) Intra-arterial saline and bismuth effects.

Procedure. Twenty rats received the usual pre-operative preparation. Of these, 10 were killed and autopsied directly and 10 were injected with N-saline followed by 30% bismuth oxychloride in 10% plasma protein solution as outlined under general methods.

Results. Gross changes in the livers are limited to a striking surface delineation of the lobular markings, while the kidneys become greyish-white in over-all appearance and show a fine white stippling on the cortical surface which represents filling of the glomeruli by the white bismuth medium. Histologically, certain well-defined artefacts present in both organs. In the liver, parenchymal cells bordering upon larger vessels containing bismuth crystals show a tendency to metachromasia and hypo-:chromatism. In the H&E sections they are revealed as pale, grey-staining areas, two or three cells deep,

surrounding the vessel, while with the Trichrome stain the areas take on a pale, grey-violaceous hue. A very similar change occurs in the kidney. Vessels of sub-arcuate (interlobular) diameter and larger are seen to be ringed by tubules in which the epi-:thelium appears pale-staining and swollen. Meta-:chromatic hypochromasia is apparent in both H&E and Trichrome sections. As determined by later studies, these changes are readily dissociated from the lesions of G.T.N. and from true liver lesions.

From the above series of preliminary investi-:gations it was concluded (1) that an adequate degree of acute hepatic centrolobular necrosis and acute renal glomerulotubular nephrosis in the rat can be attained through the use of carbon tetrachloride either singly or in combination with ethyl alcohol; (2) that the anticoagulant "phenylindanedione" produces no histo-:logical changes in either liver or kidneys; (3) that ethyl alcohol is, of itself, non-toxic to the kidneys at the designated dosage and within the prescribed time limits; (4) that the latter two drugs could thus be utilized for their respective effects without interfering with other phenomena under study;

(5) that the ganglion-blocking agent, hexamethonium, when used in the pre-agonal period at a relatively high dose produces no histological alterations in the liver and kidneys; and (6) that perfusion with saline followed by 30% bismuth oxychloride suspension produces a standard artefact which does not simulate hepatic necrosis or renal G.T.N. in any stage of development and which may be readily differentiated therefrom. Accordingly it was decided practicable to perform both histologic and arteriographic studies concurrently, utilizing the same animal for each field of investigation.

It was then decided to prosecute the investigations along the following lines: Experiment 1; Studies on carbon tetrachloride poisoning: (a) a detailed study of acute, combined carbon tetrachloride-ethyl alcohol intoxication; (b) a study of the acute-on-sub-acute combined intoxication; (c) a study of the toxic changes in sub-acute, combined tetra-:chloride-ethanol poisoning; Experiment 2; Hepatic and renal studies in acute mercury poisoning; Experiment 3; Studies on pituitrin toxicity: (a) pituitrin in the normal rat; (b) pituitrin in rats with subacute hepatic and renal damage;

(c) pituitrin in rats with acute hepatic and renal damage. Each of the above experiments represents the compiled data from two or more experimental groups containing variable numbers of rats. This has been done to greatly facilitate the presentation of material in view of the large number of animals involved. (More than 550 rats were employed in the investigations performed at Edinburgh University).

Experiment 1: Studies on carbon tetrachloride poisoning.

(a) A detailed study of acute, combined tetrachloride-ethanol intoxication.

Procedure. 159 rats comprising experimental groups A, F, G, H, I, K and Y were utilized. All but the 12 animals extracted from group A were exposed to the alcohol mixture and reduced food intake for a three-day period, following which 95 animals received carbon tetrachloride and 64 were retained as controls. The animals were then injected with the bismuth pre-:paration at various intervals as outlined under general methods. The actual intervals are detailed in Table 6.

Results. Table 6 records the interval following carbon tetrachloride, the number of rats receiving the drug, the number of corresponding controls, the number developing liver lesions, the average degree of liver damage sustained, the number developing G.T.N. and finally the average degree of severity of the G.T.N. lesion. For purposes of convenience the 1 and 2 hour intervals are merged in this Table but the lesions at these intervals are treated separately during the subsequent detailed description of the findings. As will be apparent, this Table correlates both the incidence and the severity of lesions of the liver and the kidneys with the time factor in regard to the administration of the drug.

Table 6.

Incidence & Severity of Hepatic and Renal Changes following acute CCl_4 Poisoning.

	Interval after CCl_4	No.of Rats.	No.with Hepatic Lesion	Type of[x] Hepatic Lesion	No.with G.T.N.	Degree[*] of G.T.N.
Exp.	2 hrs	7	7	Cloudy Swelling	0 (0%)	0
Cont.	0	5	0	0	0	0
Exp.	4 hrs	7	7	+,2	2 (29%)	±,1
Cont.	0	6	0	0	0	0
Exp.	6 hrs	13	13	C.S.& +,2	6 (46%)	+,1
Cont.	0	9	0	0	0	0
Exp.	8 hrs	5	5	C.S.& ++,2	5 (100%)	++,1
Cont.	0	4	0	0	0	0
Exp.	10 hrs	7	7	C.S.& ++,2	7 (100%)	++,1
Cont.	0	7	0	0	0	0

Table 6 contd.

	Interval after CCl_4	No.of Rats.	No.with Hepatic Lesion	Type of[x] Hepatic Lesion	No.with G.T.N.	Degree[*] of G.T.N.
Exp.	12 hrs	12	12	++,2	12 (100%)	++,2
Cont.	0	9	0	0	1 (11%)	++,1
Exp.	18 hrs	3	3	++,3	3 (100%)	++,2
Cont.	0	3	1(33%)	+++, 1	1 (33%)	++,1
Exp.	24 hrs	14	14	++,3	14 (100%)	++,2
Cont.	0	9	0	0	0	0
Exp.	30 hrs	6	6	++,3	6 (100%)	++,2
Cont.	0	3	0	0	0	0
Exp.	36 hrs	5	5	++,3	5 (100%)	++,2
Cont.	0	5	0	0	0	0
Exp.	48 hrs	5	5	++,2	5 (100%)	++,2
Cont.	0	1	0	0	0	0
Exp.	54 hrs	3	3	++,2	3 (100%)	++,3
Cont.	0	1	0	0	0	0
Exp.	3 days	2	2	++,1	2 (100%)	++,2
Cont.	0	1	0	0	0	0
Exp.	4 days	2	2	+,1	1 (50%)	±,1
Cont.	0	1	0	0	0	0
Exp.	6 days	2	2	±,1	0 (0%)	0
Exp.	7 days	2	1(50%)	±,1	0 (0%)	0

---

[x] Hepatic lesions, average degree: extent:

C.S. Cloudy Swelling
0 None.) 1 Patchy centrolobular
+ Metachromasia.) 2 Extensive centrolobular
± Vacuolar degeneration.) 3 Confluent centrolobular
++ Vacuolation plus necrosis.)
+++ Necrosis alone.)
++++ Early fibrosis.

[*] Renal lesions, average degree: extent:

0 None.) 1 Minimal, focal.
± Equivocal G.T.N.) 2 Marked, focal.
+ Albuminuria only.) 3 Marked, extensive.
++ Acute tubular necrosis.)
+++ Subacute G.T.N.)

Table 6 establishes an impressive correlation between hepatic and renal damage in rats subjected to carbon tetrachloride intoxication. The liver shows significant changes as early as 1 and 2 hours after the drug is administered, with a definite vacuolar, hydropic lesion developing by 4 hours. Though a small number develop "equivocal G.T.N."(i) at the 4 hour interval, significant renal changes are not observed until the 6 hour period. Thus there is a lag of about 5 hours between the onset of liver damage and the occurrence of the earliest renal lesion (principally proteinuria with the occasional accompaniment of focal, acute, tubular necrosis). These renal changes are seen in slightly less than 50% of the animals at this time; the hepatic changes will be seen to have occurred in all rats at all intervals. From 8 hours 'til the 3rd day the hepatic and renal lesions increase both in extent and severity and are found in 100% of animals. From the

---

(i)The term "equivocal G.T.N." refers to lesions in which are found scattered individual necrotic epithelial cells, occurring in haphazard fashion without any focal pattern, in excess of a basal number of dead epithelial cells representing normal "wear and tear" processes.

4th day onward the lesions in the two organs rapidly disappear, the liver damage requiring a few days longer to heal than does that of the kidney. Throughout the series there is noted a good degree of correlation between the severity of the hepatic and the renal lesions.

Of the 64 control animals, only 2 show the presence of a renal lesion identifiable as minimal, acute G.T.N., one of which is noted to have an associated patchy, centrolobular necrosis of the liver.

Morbid anatomy and histology.

Macroscopic. The liver shows a progressive increase in lobular markings, particularly obvious following bismuth injection. There is decreased filling of sinu-:soidal vessels from 1 hour to 24 hours, accentuating the portal tracts by naked-eye observation. Prior to injection the liver appears slightly more hyperemic than normal in the early intervals. Beyond 24 hours it takes on a blotchy, stippled appearance due to tiny centro-:lobular hemorrhagic zones. It appears grossly normal by the 4th or 5th day.

The kidneys show no naked-eye alterations throughout the intervals under observation. There is no increase in weight such as is observed in mercury poisoning in the later stages.

Microscopic. Histological alterations are found in the liver as early as one hour after the drug is administered. This earliest change consists of cloudy swelling and pallor of the cord cells in the inner two thirds of the liver lobules. Injected bismuth is seen to penetrate the outer third of the lobules, but is excluded from the remainder (fig. 54). A normal injected control liver is shown for contrast in fig. 55.

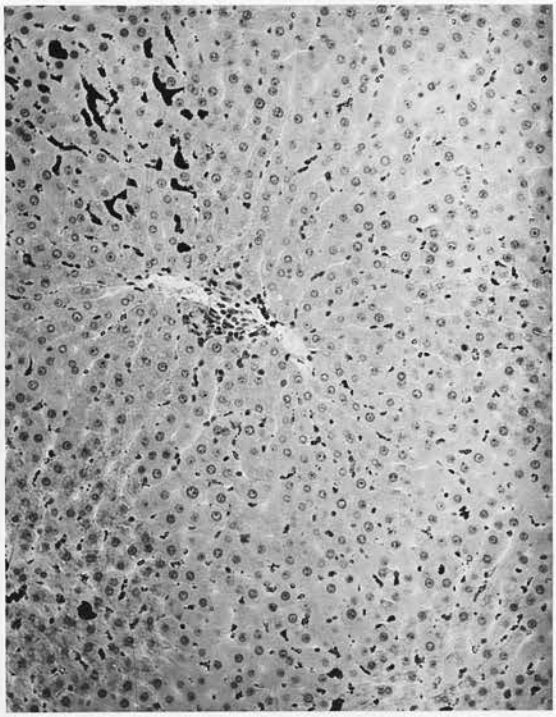


Fig.54. No.D-95. Rat liver, H&E, x150. 1 hr after carbon tetrachloride. Cloudy swelling of the cord cells with obliteration of the sinusoids in the inner two thirds of the lobules. Note exclusion of bismuth crystals in this zone.

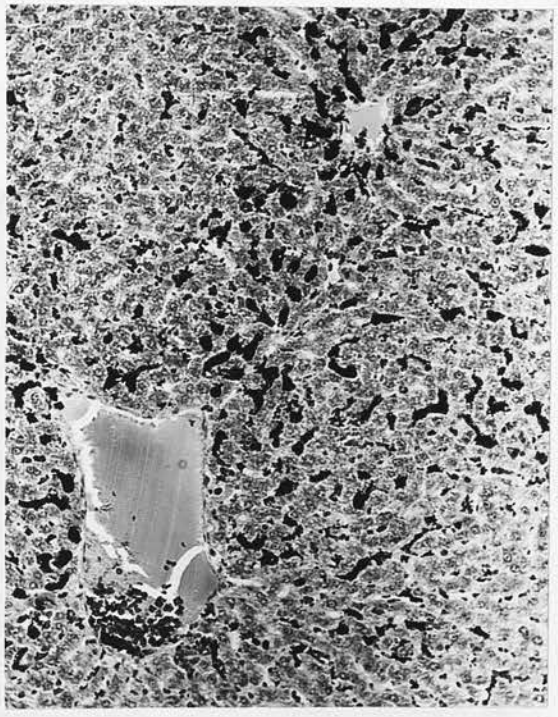


Fig.55. No.A-10. Rat liver, H&E x150. Normal control liver showing glycogen-laden cord cells and bismuth penetration throughout the sinusoids of the entire lobule.

The pallor of these affected cells is noted to increase by 2 hours, but the liver is otherwise not remarkable. At the 4 hour interval, occasional necrotic cells and a minimal leukocytic infiltration are seen (fig. 56), while the boundary between the outer third and inner two thirds is sharply demarcated by very pale, swollen hydropic cells, usually about one cell in depth, fre-:quently displaying nuclear hyperchromatism. Between 8 and 10 hours frankly necrotic cells are found in larger numbers within the affected zone, most common adjacent to the central vein. A far more pronounced leukocytosis is seen (fig. 57).

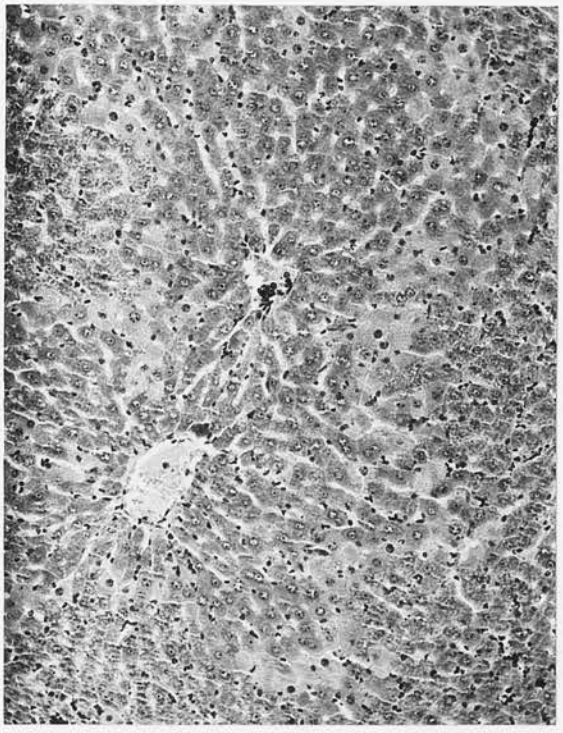


Fig.56. No.A-80.Rat liver, H&E x150. 4 hrs after carbon tetrachloride. Note the pale and swollen cells of the inner ⅔s of lobules, bounded by vacuolated, hydropic cells forming a sharp margin to the damaged inner zone.Note also scattered necrotic cells, plus early leukocytic reaction.

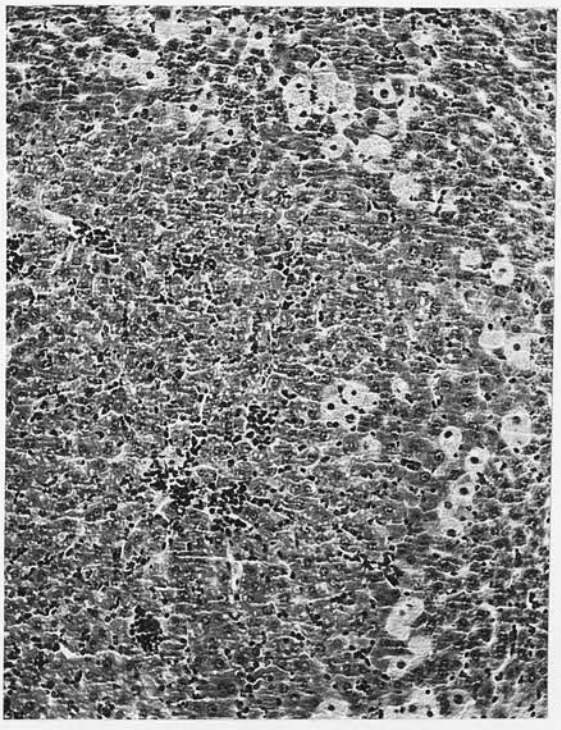


Fig.57. No.A-86. Rat liver, H&E x150. 8 hrs after carbon tetrachloride. Note the increase in both necrosis and leukocytosis. The hydropic boundary remains in very distinctive fashion.

Within 24 hours, active resolution of necrotic cells is seen in association with some degree of parenchymal collapse. The large hydropic cells remain very prominent at the junction of the inner two-thirds and outer third of the lobules (fig. 58), and there is early diapedesis of erythrocytes in the necrotic central zone. After 48 hours, bismuth injection compound is found in the necrotic central vein areas, indicating re-establishment of the circulation (fig. 59). There is stromal collapse, hemorrhage and mitotic activity.

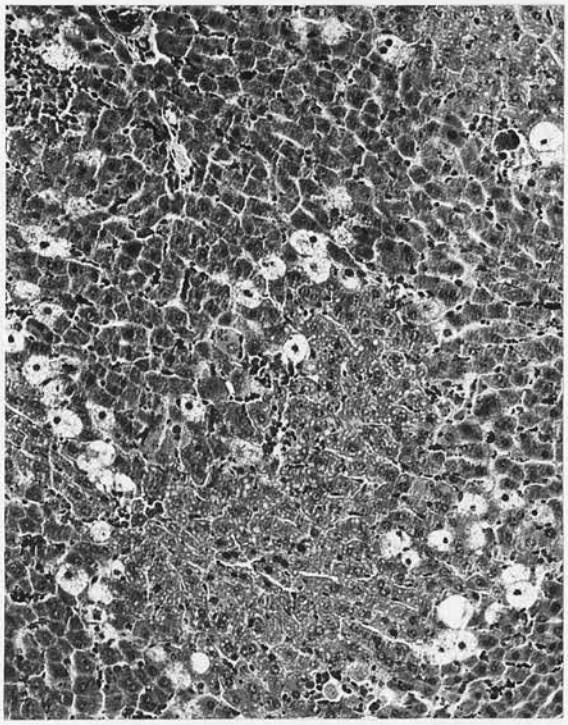


Fig.58. No. A-19. Rat liver H&E x150. 24 hrs after carbon tetrachloride. The central zone of the lobule shows extensive necrosis and a slight, early degree of coll-:apse.There is incipient dia-:pedesis of erythrocytes into the centre of the lobule, though the bismuth is confined to the periphery.

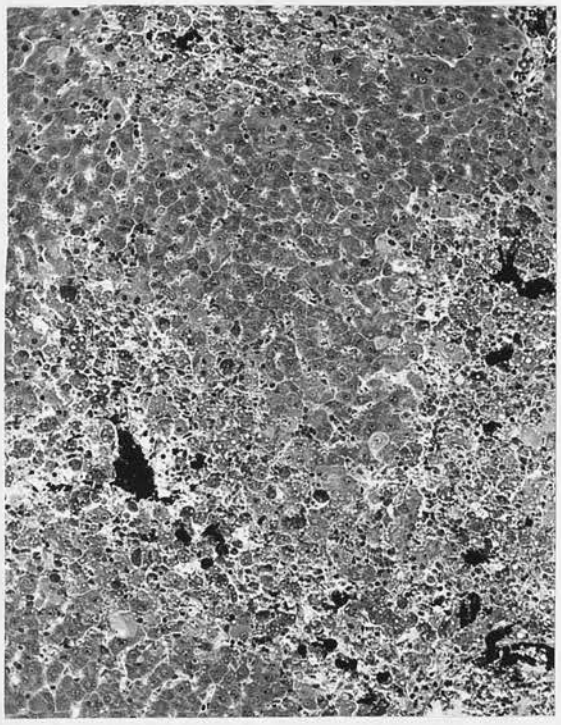


Fig.59. No. A-1. Rat liver, H&E x150. 48 hrs after carbon tetrachloride. The bismuth now floods the necrotic central vein areas. Note the increased amounts of stromal collapse and hemorrhage.

On the third day most of the debris is cleared away. Numerous dark cells remain in the formerly necrotic areas and parenchymal condensation is a rather marked feature. Kupffer cells are prominent throughout the lobule (fig. 60). After one week scattered pyknotic nuclei, occasional binucleate cells and minimal cytoplasmic polychromasia are the only remnants of the necrotizing process (fig. 61). Mitosis is apparent throughout the entire period of intoxication and repair.

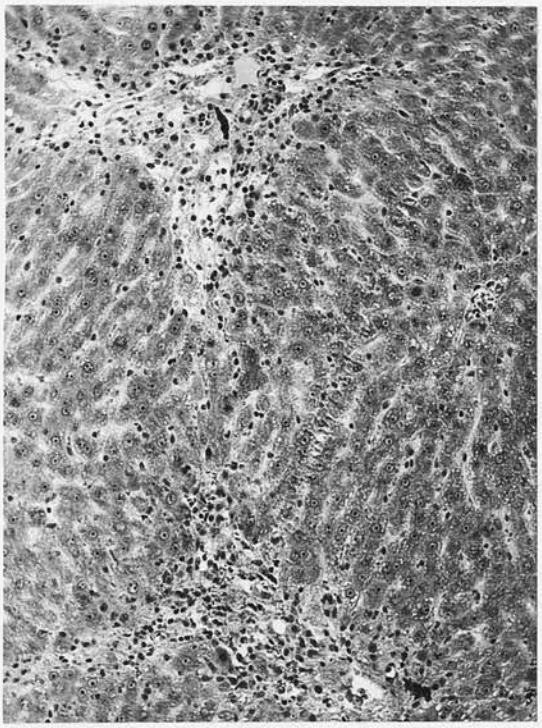


Fig.60. No.62. Rat liver, H&E x150. 3 days after carbon tetrachloride.Marked collapse of the parenchyma, numerous "dark cells" and prominent Kupffer cells are apparent.

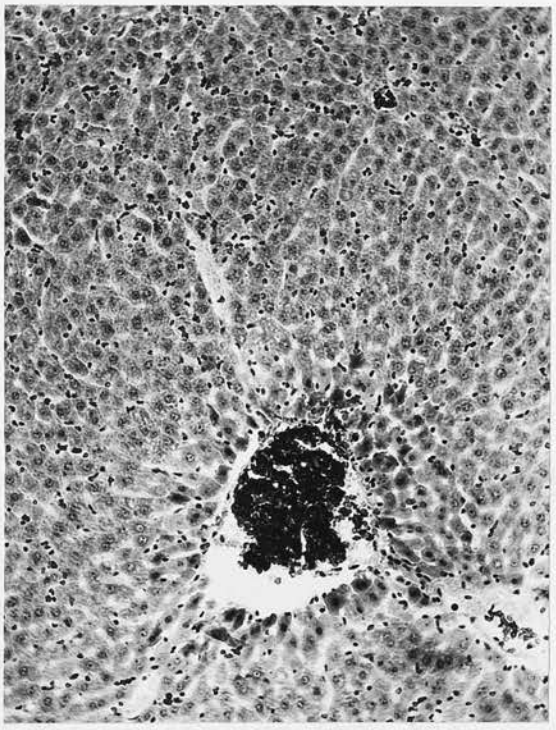


Fig.61. No.70. Rat liver, H&E x150. 7 days after carbon tetrachloride. The lesion is practically healed.Scattered pyknotic nuclei, some cytoplas-:mic metachromasia and occas-:ional binucleate cells are the only remains.

The earliest demonstrable renal lesion is seen at 6 hours, 5 hours after histological alterations appear in the liver. There is minimal impairment of glomerular permeability with small amounts of protein material lying free in the capsular spaces and occasional pale green casts in the loops of Henle as seen with the trichrome stain. The glomerular changes are probably significant but not diagnostic since the kidneys have been injected and the permeability may well be to the foreign protein carrier. Pale, homogeneous protein casts in tubules are a significant finding, however, and help in the assessment of the glomerular changes. The characteris-:tic tubular changes of acute G.T.N. as described in Sections II and III are found in 3 of the 6 rats with renal changes, in scattered foci, involving proximal and distal convoluted tubules alike. These changes consist of acute, smudgy cytoplasmic deterioration and nuclear pyknosis in the epithelial lining cells plus intraluminal deposits of sloughed, necrotic epithelium. Cloudy swelling is an accompanying finding, character-:ized by stellate intrusion of the epithelium into the tubal lumina. The above features are illustrated in figs. 62 and 63, 6 and 12 hours after carbon tetra-:chloride respectively. At the 12 hour interval a focal vacuolar degeneration of the proximal convoluted

tubular epithelium has developed in 100% of the animals. This feature is well shown, in conjunction with frank necrosis, in figs. 64, 65 and 84. The vacuolar change consists of enormous hydropic swelling of the cytoplasm, associated with smudgy or granular mitochondrial degeneration and often with rupture into the lumen.

Fig.62. No.A-82.Rat kidney, Tri. x300. 6 hrs after carbon tetrachloride. Focal acute degenerative changes in proxi-:mal and distal tubular epi-:thelium, with cloudy swelling in some of the proximal tubules and protein exudate in one of the capsular spaces.

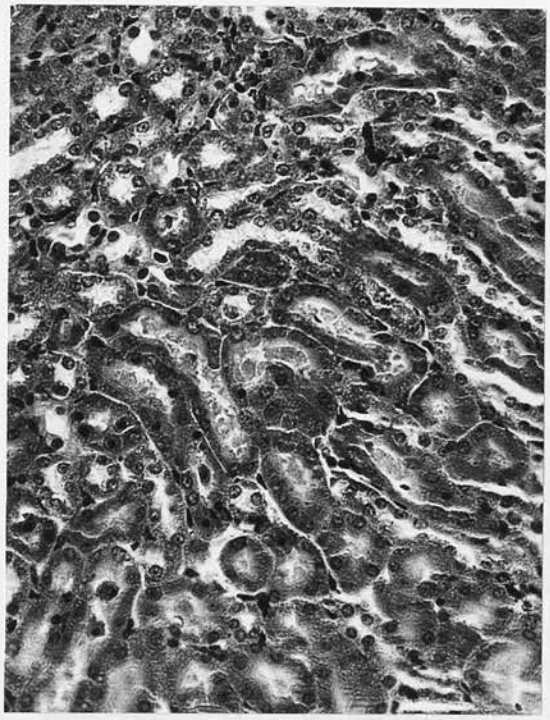


Fig.63. No.A-34. Rat kidney, H&E x300. 12 hrs after carbon tetrachloride. The focal changes are more widespread and more advanced than in the previous figure. Numerous sloughed epi-:thelial cells are seen in tubal lumina.

This lesion characterizes rat carbon tetrachloride G.T.N., but is not a dominant feature of the various forms of G.T.N. described in human and rabbit kidneys (Sections I and II). Fig.34 displays the lesion to a mild degree in bile nephrosis. It persists in increasing severity and distribution from 12 to 54 hours after intoxication, but is absent in specimens viewed at 72 hours and later.

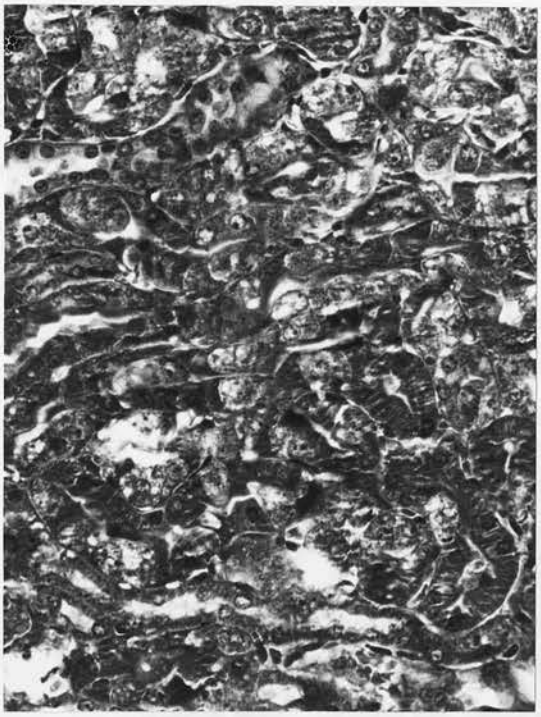


Fig.64. No.A-1, Rat kidney, Tri.x300. 48 hrs after carbon tetrachloride. Cells of the proximal tubules showing extreme hydropic swelling plus mitochondrial degeneration. Note evidence of desquamation and cast formation.

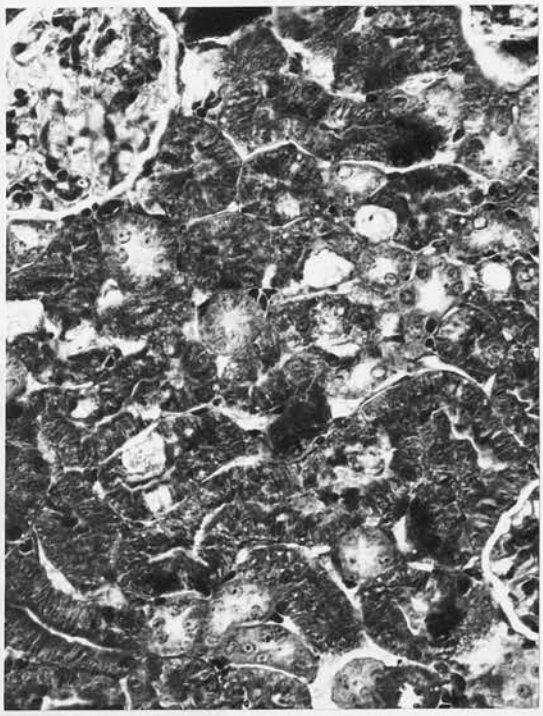


Fig.65. No. A-3, Rat kidney, Tri.x300. 54 hrs after carbon tetrachloride. The features are very similar to those seen in fig. 64, though less marked.

Within 24 hours, protein and cellular casts are seen in the collecting tubules and elsewhere throughout the nephron (fig.66). No changes are found in the blood vessels or in the interstitium. The renal lesion is completely healed by the 4th day, leaving no sign of former damage.

Sections of the liver and kidney from 2 animals showing hydropic degeneration (A 86, fig.57 and A-1, fig. 64) were stained by the periodic acid-Schiff routine according to McManus, 1948, and revealed complete absence of polysaccharide material in the vacuolated cells of both organs.

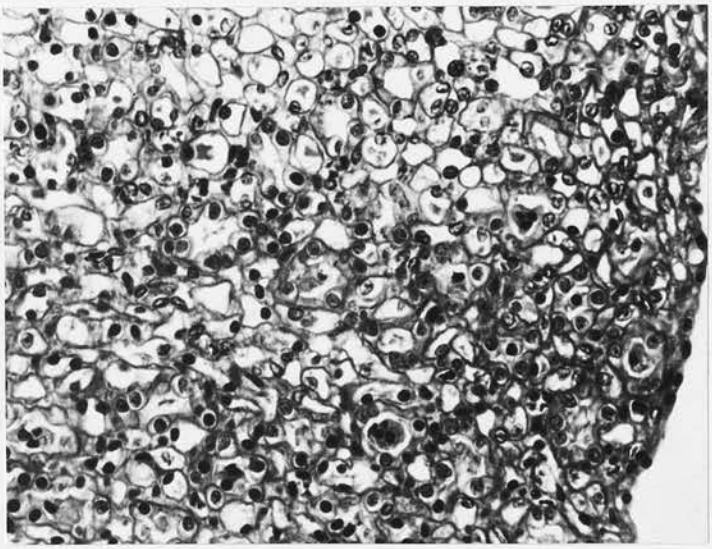


Fig.66. No.97. Rat kidney, Trichrome x300. 24 hrs after carbon tetrachloride. Protein and cellular casts in the collecting tubules.

(b) A study of acute-on-subacute combined intoxication.

Procedure. 36 rats comprising experimental groups O & P were utilized. All received the ethanol mixture and reduced food intake for 3 days, following which two successive anesthetic doses of carbon tetrachloride were given 4 days apart. Five days after the second dose of CCl_4, 23 rats were given a 3rd dose of the drug. The remaining 13 rats were retained as controls and form the substance of Experiment 1(c). Following the 3rd dose of drug the animals were sampled at varying intervals as indicated in Table 7. In this Table, composed as was Table 6, with minor exceptions, the control animals have been entered as "subacute" to conform with the third part of this experiment.

Results. Morbid anatomy and histology.

Macroscopic. The liver appears more congested than normal and shows prominence of lobular markings. The kidneys appear somewhat hyperemic but show no change of weight or other remarkable features.

Microscopic. The hepatic lesions are far more massive than those produced by a single dose of carbon tetrachloride. Evidence of the prior hepatic insults is seen in the region of the central vein. Twenty-four hours after the third dose of drug there appears some

slight increase in centrolobular fibrous tissue as seen with the trichrome stain. The acute changes are readily observed in the central and middle zones of the lobule (fig. 67). Three days after the 3rd dose of drug, definite fibrosis and marked proliferation of new bile ducts is found (fig. 68). A slight fatty metamor-:phosis is seen throughout the interval.

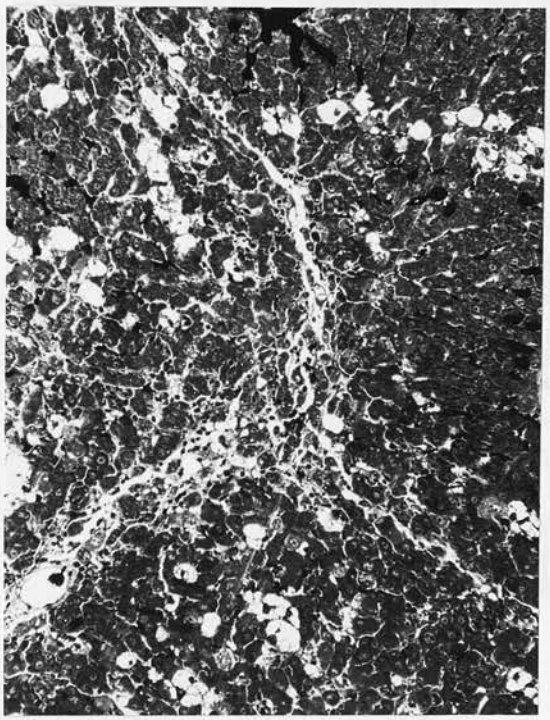


Fig.67. No.B-64. Rat liver, Tri.x150. 24 hrs after 3rd dose of CCl_4. Acute nec-:rosis superimposed on paren-:chymal collapse and slight central fibrosis.

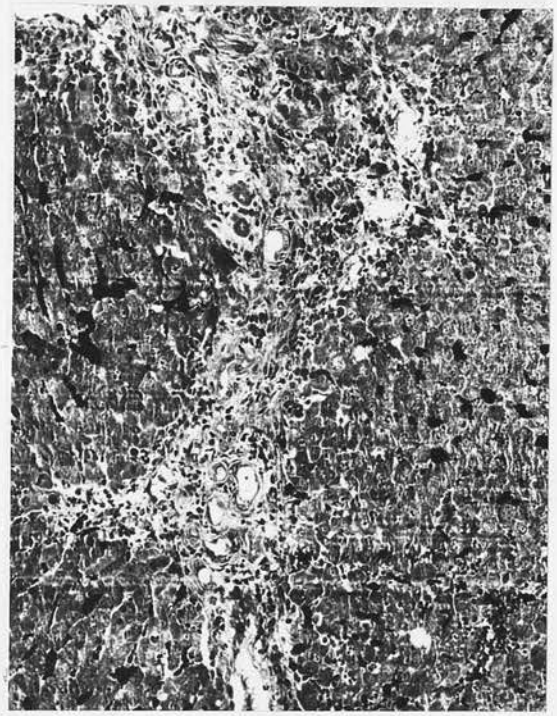


Fig.68. No. B-69, Rat liver, Tri.x150. 3 days after 3rd dose of CCl_4. Fibrosis and proliferation of bile ducts.

The renal lesion is a massive, acute and subacute facsimile of the single dose carbon tetrachloride lesion in the rat. The tubular lesion is a very extensive acute necrosis and vacuolar hydropic degeneration of the epithelium (fig. 69).

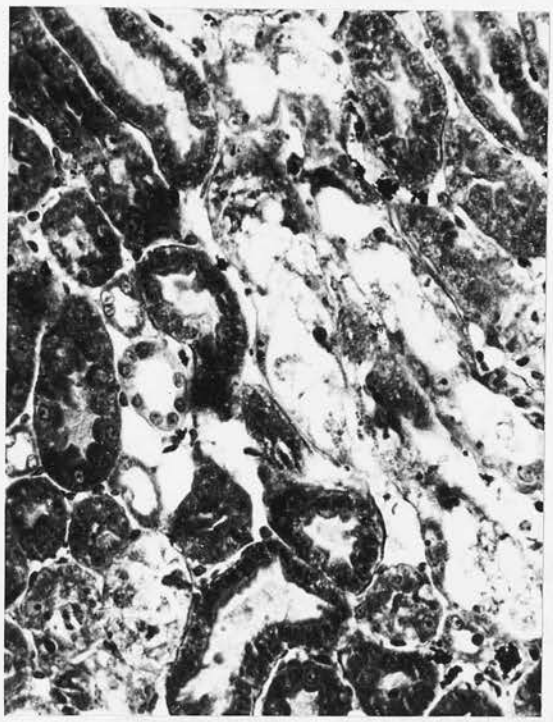


Fig.69. No. B-69. Rat kidney, Tri.x300. 3 days after 3rd dose of CCl_4. Extensive acute and sub-acute G.T.N. (necrosis) and hydropic degeneration of tubular epithelium.

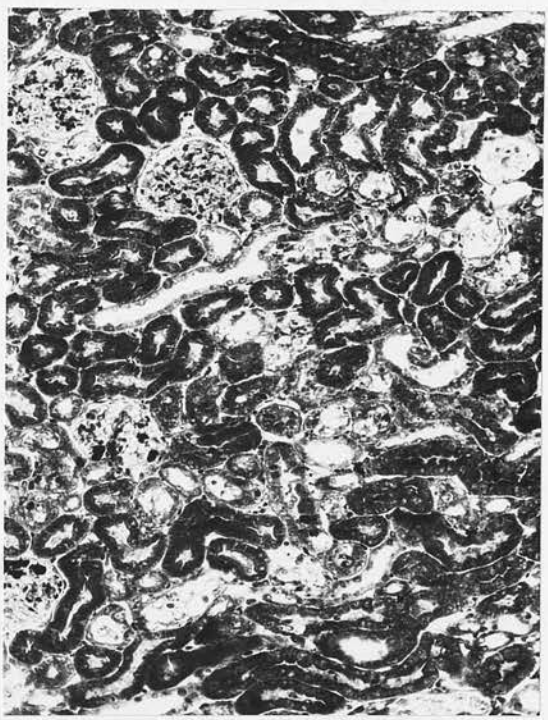


Fig. 70. No.B-69. Rat kidney, Tri.x150. 3 days after 3rd dose of CCl_4. Low-power view of same kidney as fig. 69, to orient with regard to both distribution and extent of the lesion.

Tubulorrhexis is absent, the interstitial tissues appearing normal. The lesion is situated predominantly in the cortical region, with extension to the cortico-medullary zone via finger-like processes analogous to medullary rays in humans (fig. 70).

These rays divide the rat kidney into numerous micro-:scopic lobules, having at their centres the inter-(or intra)-lobular arteries. The acute changes are observed 5 hours after the last dose of drug, a time very similar to that found in the single-dose lesion. There is slight fatty degeneration which occurs pre-:dominantly in the proximal convoluted tubules of the cortico-medullary zone in both experimental and control animals throughout the intervals studied, forming a feature of subacute G.T.N.

The analysis of the data, presented in Table 7, will be found along with the table, following the presentation of Experiment 1(c).

(c) A study of the toxic changes in subacute, combined tetrachloride-ethanol poisoning.

Procedure. Thirteen rats, comprising the control material from Experiment 1(b) (groups O & P), were employed. These rats had received the ethanol mixture for 3 days, followed by carbon tetrachloride administration on two occasions, 4 days apart. They were subsequently sampled 5, 6, 7 & 8 days after the last dose of drug, as detailed in Table 7.

Results. Morbid anatomy and histology.

Macroscopic. The liver is mildly congested and the lobular markings are prominent. The kidneys appear slightly more hyperemic than normal but are not otherwise noteworthy.

Microscopic. In the liver the residuum of former necrosis is somewhat greater than that seen at a comparable period following a single necrotizing dose of CCl_4, while regenerative changes are slightly more retarded. The dominant features at 6 days consist of extensive metachromasia and moderate pleomorphism of the inner third of the lobule plus prominence of cells of the reticulo-endothelial system. Numerous isolated necrotic cells are scattered throughout the inner lobular zone. By the 9th day the liver appears entirely healed.

In the kidneys, rare foci of necrotic tubules plus very occasional protein and cellular casts in the distal and collecting tubules are noted on the 6th day following the 2nd necrotizing dose. By the 9th day the kidney is again completely normal. The renal picture at these intervals corresponds well with the alterations observed in the respective livers.

Table 7 displays the findings of Experiment 1,(b & c), from the temporal-incidence-severity bias.

Table 7.

Incidence & Severity of Hepatic and Renal Changes after multiple doses of CCl_4.

	Time after last CCl_4.	No.of Rats.	No.with Hepatic Lesion	Type of[x] Hepatic Lesion	No. with G.T.N.	Degree of[*] G.T.N.
Ac-on-sub.	3 hrs	4	4	Cloudy Sw. & ++++,2;	4	+++,1
Subacute.	6 days	3	3	+,2 & regen.;	2	+++,2
Ac-on-sub.	5 hrs	2	2	++,3 & sl.fat;	2	++,2 &+++,1
Subacute.	6 days	2	2	+,2 & sl.fat;	2	+++,2
Ac-on-sub.	12 hrs	2	2	++,3 & ++++,1;	2	++,3
Subacute.	6 days	2	2	+,2 & mod.fat;	2	+++,1
Ac-on-sub.	24 hrs	4	4	++,3 & ++++,1;	4	++,3
Subacute.	7 days	2	2	+,2 & mod.fat;	2	+++,1
Ac-on-sub.	30 hrs	4	4	++,3 & ++++,1;	4	++,3
Subacute.	7 days	2	2	+,2 & mod.fat;	2	+++,1
Ac-on-sub.	48 hrs	4	4	+++, 3 & ++++,2;	4	++,2 &+++,1
Subacute.	8 days	1	1	±,1 & sl.fat;	1	+++,1
Ac-on-sub.	72 hrs	3	3	+++,2 & ++++,2;	3	+++,2
Subacute.	9 days	1	0	0 (sl.fat);	0	0

---

[x] Hepatic lesions, average degree: extent:

C.S.	Cloudy Swelling		
0	None.	1	Patchy centrolobular
±	Metachromasia.	2	Extensive centrolobular
+	Vacuolar degeneration.	3	Confluent centrolobular
++	Vacuolation plus necrosis.		
+++	Necrosis alone.		
++++	Early fibrosis.		

[*] Renal lesions, average degree: extent:

0	None.	1	Minimal, focal.
±	Equivocal G.T.N.	2	Marked, focal.
+	Albuminuria only.	3	Marked, extensive.
++	Acute tubular necrosis.		
+++	Subacute G.T.N.		

From Table 7 it is again apparent that a close correlation between liver damage and renal G.T.N. exists, both on an over-all basis and in regard to the relative severity of the lesions in the two organs. Inter-:comparison of Tables 6 and 7 reveals that repeated intoxication with carbon tetrachloride retards the reparative processes in both liver and kidneys and increases both the severity and the distribution of the lesions in these organs. Thus subacute G.T.N. is found in the kidneys of rats more than a week after the last contact with CCl_4 in the multi-dose group, whereas the lesion has disappeared from the kidneys by the 3rd or 4th day after a single dose of the drug. The renal and hepatic lesions after the 3rd dose of poison are seen to be much more widely distributed and to show a greatly intensified degree of severity over their single-dose equivalents. In the multiple-dose experiments there is seen a slight to moderate fatty degeneration of both hepatic cord cells and proximal convoluted tubular epithelium. It is also apparent that the acute renal lesion does not appear appreciably earlier in animals with subacute hepatic damage than in normal animals, but that the rate of its disappearance is markedly delayed.

Summary of the findings in Experiment 1.

1. There is an immediate toxic reaction of liver cord cells situated in the inner ⅔s of the lobule, which, in 1 hour appear pale and swollen and show obliteration of the sinusoidal pattern with the exclusion of bismuth injection compound in this zone.

2. An hydropic degenerative change occurs in a thin rim of cells at the junction of the inner ⅔s and outer ⅓ of the lobule, quite distinct at 4 hours and later.

3. The renal changes of incipient, acute G.T.N. appear after 6 hours; i.e. there is a 5 hour delay between the appearance of toxic effects in the liver and in the kidneys.

4. Well-marked necrosis and avascularity of the central portion of the liver lobule are seen after 8 to 10 hours, the avascularity persisting beyond 24 hours.

5. Pooling of bismuth is seen in the necrotic zone by 48 hours.

6. A vacuolar or hydropic degeneration of cells of the proximal convoluted tubular epithelium first appears at 12 hours and increases in degree and distribution up to 54 hours.

7. Complete healing of the single-dose lesion takes place, in the liver after 1 week and in the kidneys after 4 days.

8. A delay of several days in the reparative processes in both organs follows two or more doses of the drug. (Subacute phase).

9. There is no change in the time of onset of the acute lesion in either liver or kidneys after a third dose of drug. (Acute-on-subacute phase).

10. Production of early cirrhotic changes (fibroblast and bile-duct proliferation) occurs in the liver after a 3rd dose of drug.

11. Mild fatty degeneration of hepatic cord cells and proximal convoluted tubular epithelium characterizes the subacute phase.

12. There is a 100% correlation of combined liver and kidney damage between time intervals of 8 and 72 hours after administration of a single dose of the drug.

13. A 100% correlation of combined liver and kidney damage also exists between intervals of 8 hours and 8 days following multiple-dose administration.

14. The spontaneous occurrence of acute G.T.N. not associated with hepatic disease occurs in 1 out of 64 control animals and the spontaneous occurrence of liver necrosis with an accompanying acute G.T.N. is seen in another 1 of the 64 controls.

15. The vacuolar hydropic change in both liver and kidneys is shown to be free from polysaccharide material as estimated by the P.A.S. stain.

Experiment 2; hepatic and renal studies in acute mercury poisoning.

Procedure. A total of 59 rats were employed in this investigation, 32 experimental and 27 controls, comprising experimental groups J, L & Z. The animals were prepared by a three-day exposure to the ethanol-anticoagulant mixture but were allowed to eat an otherwise normal diet ad lib.. Thirty-two rats were then given 0.5 mgs of mercuric chloride (0.1 cc of 0.5% W/V $HgCl_2$) intramuscularly in the thigh. Variable numbers were sampled from the poisoned group and the

normal controls during a series of subsequent periods. All animals then underwent injection with saline and bismuth as outlined under general methods.

Results. The correlation between time of onset, incidence and severity of the hepatic and renal changes is detailed in Table 8.

Table 8.

Incidence and Severity of Hepatic and Renal Changes Following $HgCl_2$

Time after $HgCl_2$	No. of Rats	No. with Hepatic Lesion	Type of Hepatic Lesion*	No. with Renal Lesion	Type of Renal Lesion*
Expt. 1 hr	2	2	a.	0	0
Cont. 0	2	0	0	0	0
Expt. 2 hrs	4	4	a.	0	0
Cont. 0	2	0	0	0	0
Expt. 4 hrs	2	2	a.	0	0
Cont. 0	2	0	0	0	0
Expt. 6 hrs	6	6	a,b,c.	5	++,1
Cont. 0	4	0	0	0	0
Expt. 9 hrs	2	2	a,b,c.	2	++,1
Cont. 0	2	0	0	0	0
Expt. 1 day	5	3	b,c.	5	++,3
Cont. 0	4	0	0	0	0
Expt. 2 days	2	0	0	2	++,3
Cont. 0	2	0	0	0	0
Expt. 3 days	2	0	0	2	++,3 reg.
Cont. 0	2	0	0	0	0
Expt. 5 days	2	0	0	2	+++,3 reg.
Cont. 0	2	0	0	0	0

Table 8 contd.

Time after $HgCl_2$	No.of Rats	No.with Hepatic Lesion	Type of[x] Hepatic Lesion	No.with Renal Lesion	Type of[*] Renal Lesion
Expt. 6 days	2	0	0	2	+++,2, reg.
Cont. 0	2	0	0	0	0
Expt. 7 days	2	0	0	2	reg.
Cont. 0	2	0	0	0	0

---

[x] Liver lesion: a. = swelling of cord cells plus sinusoidal obliteration.
b. = granular mitochondrial degeneration.
c. = hydropic degeneration.

[*] Kidney lesion:

++ = acute tubular necrosis. 1. = patchy, focal.
+++ = subacute tubular necrosis. 2. = widespread, focal.
reg. = regeneration. 3. = generalized.

From Table 8 it is apparent that an immediate hepatic lesion occurs with mercurial intoxication, which is associated within 5 hours with a renal lesion identical to the minimal, non-specific lesion of acute G.T.N. as described in Sections II and III, and in Experiment 1 of Section IV. There is an 83% correlation between liver and kidney lesions at the 6 hour interval and a 100% correlation at 9 hours. At 24 hours, the liver lesions are reduced in incidence and severity while the renal lesions have become severe and widespread, particularly in the proximal convoluted

tubules. It is also apparent that the type of renal damage has changed from the non-specific variety as seen at 6 and 9 hours to a different and very specific, severe form in the 24 hour specimen. In the first 9 hours the findings, both in the liver and the kidney bear a peculiar resemblance to the changes in carbon tetrachloride poisoning.

Morbid anatomy and histology.

Macroscopic. Acute edema occurs almost immediately at the site of injection in the thigh, resulting in a swollen and painful lower extremity. The leg appears normal within 24 hours.

The liver appears somewhat hyperemic in-situ. Upon injection with the bismuth medium, the lobular markings are accentuated due to pallor of the intervening tissues, during the 1st 9 hours. From 20 hours on, this gross change is no longer detectable.

The kidneys appear somewhat congested from the capsular and cut surfaces during the 1st 24 hours, but they show no increase in weight. By 48 hours there is found a 35 to 40% increase in the weight of each kidney, this weight increase being maintained throughout the remainder of the week at a fairly constant level.

A small number weigh within normal limits by the 7th day. During these later periods the kidneys are large, hyperemic and bulge from their cut surfaces.

Microscopic. In the liver, careful examination reveals congestion of the larger vessels and a swelling of the parenchymal cells throughout the entire lobule which causes an apparent diminution in the size of the simusoids and gives the microscopic field a solid, cellular appearance. Figs 71 and 72 illustrate a normal control and a 2 hour mercurial liver respectively.

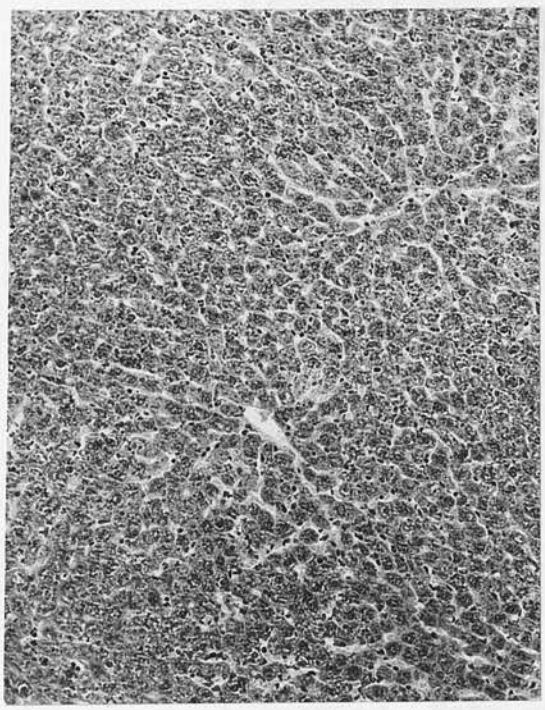


Fig.71. No.A-62. Rat liver, H&E x150. Normal control.

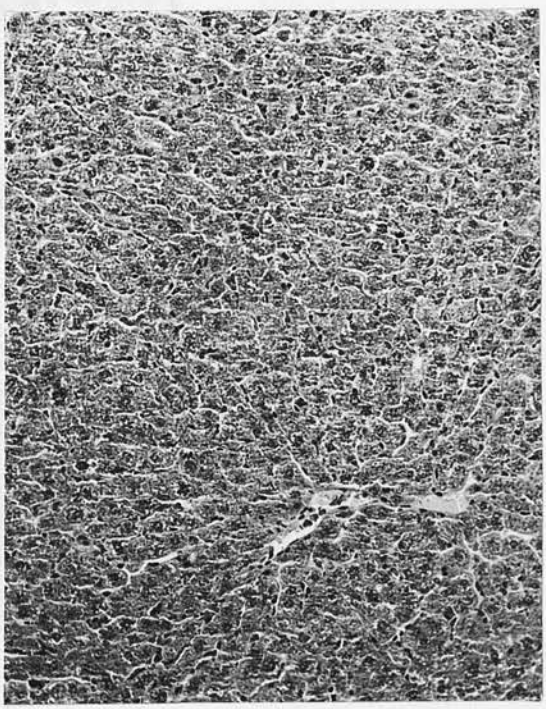


Fig.72. No.A-58. Rat liver, H&E x150. 2 hours after mercury poisoning. Note obliteration of the sinusoids by swollen cord cells. The field appears unusually cellular.

The swelling is seen as early as 1 hour following mercury intoxication and it progresses to a coarsening of the mitochondrial elements, to the occasional formation of perinuclear vacuolization and to a form of hydropic degeneration between 6 and 24 hours (fig.73). The swelling of the cord cells starts to subside by 9 hours and a normal sinusoidal pattern can be made out by 24 hours. The hydropic change is absent within 48 hours.

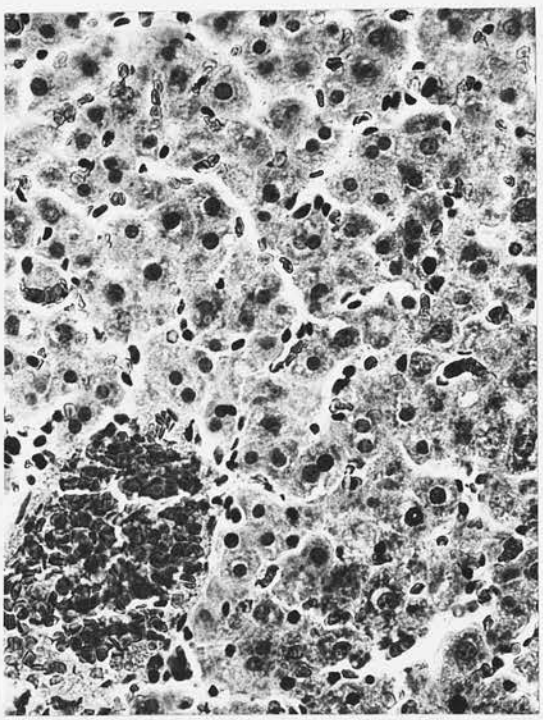


Fig.73. No.A-66. Rat liver, H&E x300. 6 hours after mercury poisoning. Note granular mitochondrial change in cytoplasm, perinuclear vacuoles and hydropic degeneration of scattered cells.

At this interval there is a numerical increase in the so-called "dark cells" and binucleate cells are more

common in this and subsequent specimens. A rare mitotic figure is present in specimens taken between 9 and 48 hours.

In the kidney the first demonstrable change occurs 6 hours after the intoxication. This consists of glomerular hyperemia and very early, minimal, focal, acute degenerative changes in the convoluted tubules, affecting proximal and distal alike. These non-specific degenerative changes consist of smudgy cyto-:plasmic coagulation, slight cloudy swelling and nuclear pyknosis of small groups of epithelial cells in haphazard distribution both in the individual tubules and through-:out the entire thickness of the cortex. This lesion appears identical to that described as minimal, acute G.T.N. in the rat with CCl_4 intoxication, (Experiment 1, Section IV). No specific changes can be traced in the renal histology at this time.

By 20 hours, a decided alteration is seen in the epithelia of the proximal and distal convoluted tubules, distributed in a very extensive fashion throughout the cortex. There is total coagulative necrosis of the vast majority of nephrons, sparing isolated clumps of tubules. In roughly 50% of the animals there would appear to be some tendency to select the cortico-medullary zone

(fig.74), but in the remainder the lesion is spread diffusely throughout the cortex. The epithelium of the proximal and, to a lesser extent, of the distal convoluted tubules is totally disrupted and anucleate. In some instances, disruption of the basement membranes can be seen. All tubules are filled with cellular and amorphous casts.

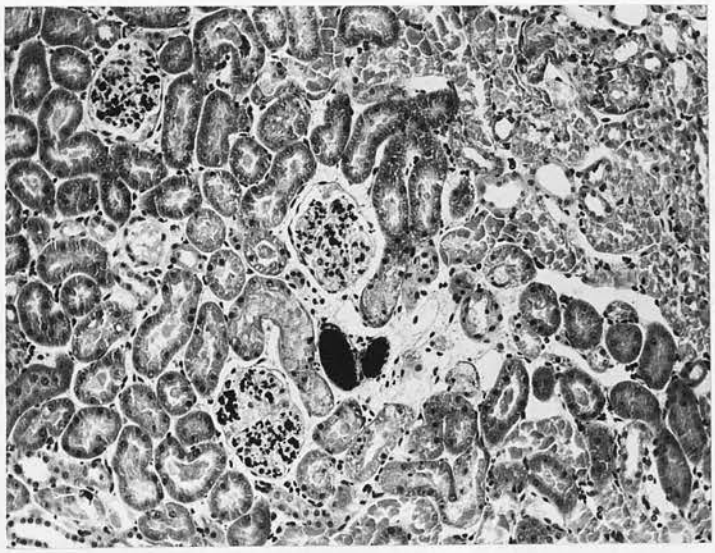


Fig.74. No. A-92. Rat kidney, H&E x150. Low-power field to show the tendency to a cortico-medullary distribution of the necrotizing process 20 hours after mercury poisoning.

The lesion spares the descending loop of Henle and the collecting tubules. Vascular and interstitial tissues appear normal, apart from the glomeruli, where most capsular spaces contain amorphous and granular debris.

After 48 hours the histological features remain very much as before, but evidence of active repair presents in the form of widespread mitosis, situated in the spared segments of the proximal and distal convoluted tubules (fig. 75). By the 3rd day most of the tubules have been relined by a low-cuboidal, basophilic epithelium (fig. 76). The tubal lumina remain plugged with necrotic epithelial slough and protein casts.

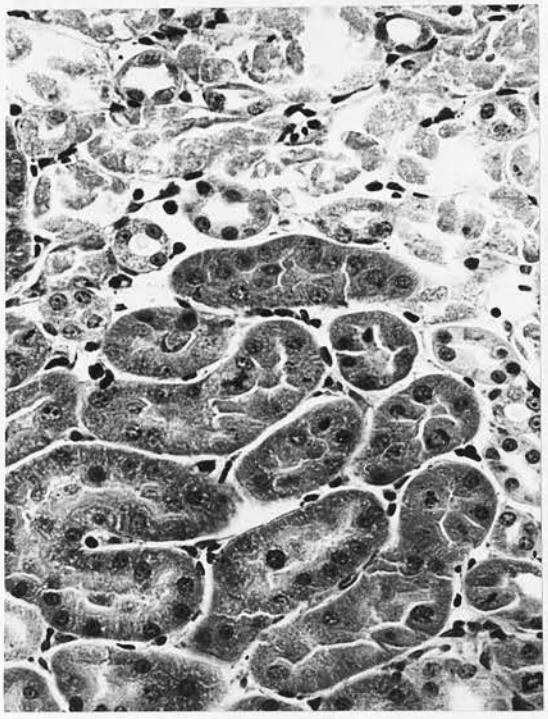


Fig.75. No.A-96, Rat kidney, H&E x300. 48 hrs after mercurial poisoning. Note the numerous mitotic figures.

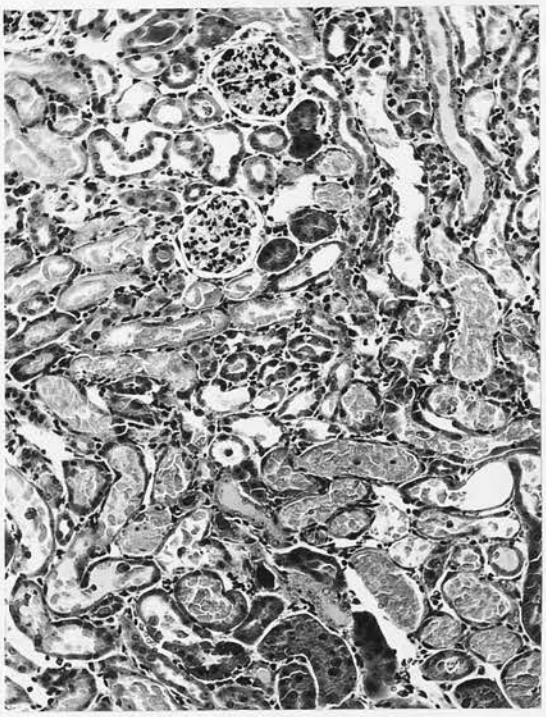


Fig.76. No.B-2, Rat kidney, H&E, x150. 3 days after mercury. Extensive re-epi-:thelialization with a low-cuboidal, basophilic cell.

The damaged tubules show an apparent dilatation, due, in part, to the thinness of the regenerated epithelium. There is now present interstitial edema and early infiltration of chronic inflammatory cells. A smudgy fibrinoid necrosis of the walls of arteries of large and small dimensions is seen from 24 to 48 hours, though no hemorrhage from necrotic vessels can be made out (fig. 78).

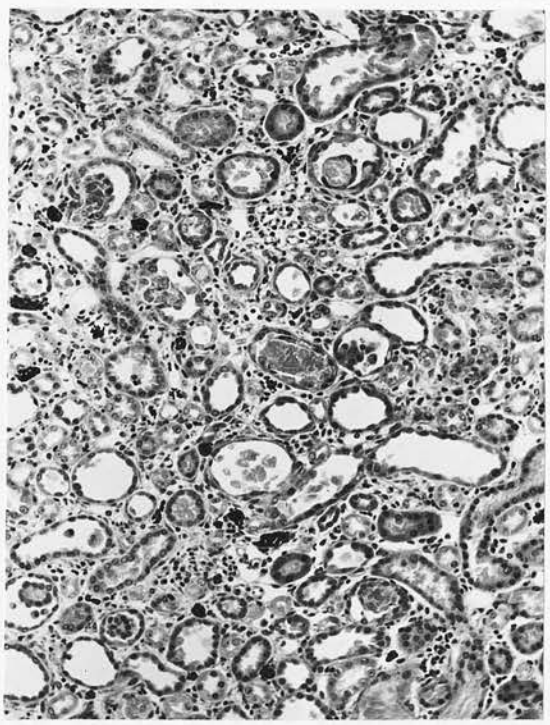


Fig.77. No.B-9. Rat kidney, H&E x150. 6 days after mercur-:ialization. Note heaping-up of regenerating epithelium and deposition of calcium salts. Sites of former tubulorrhexis, with marked interstitial inflammatory changes.

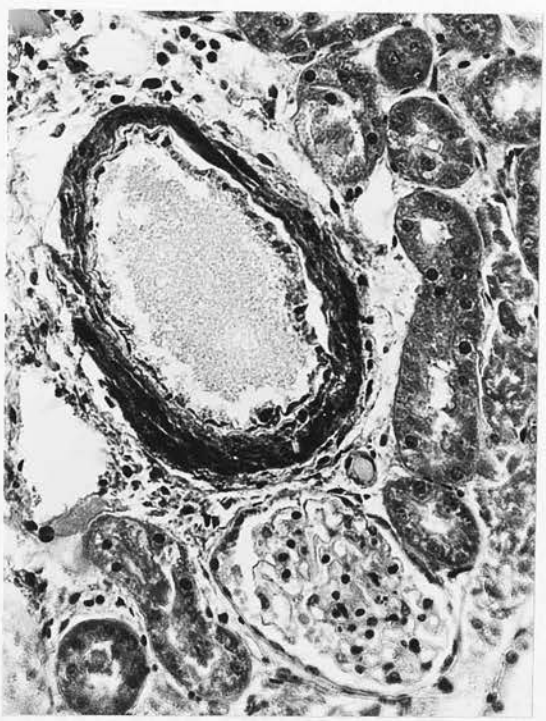


Fig.78. No.A.96. Rat kidney, Tri.x300. 48 hrs after mercury poisoning. Note the fibrinoid necrosis of the wall of the arcuate artery.

From the 5th to the 7th day reparative processes continue to dominate the picture. Much debris remains to be cleared; many tubules show abortive attempts at regeneration, with heaped-up epithelium and surrounding chronic inflammatory cell infiltration of the interstitium representing areas of former tubulorrhexis; and calcium salts are deposited on casts and within epithelial cells (fig. 77). No fatty degeneration is seen in this series.

Summary of the findings in Experiment 2.

1. There is an immediate swelling of liver cord cells throughout the lobule, producing marked obliteration of the sinusoidal pattern.

2. Five hours after the liver lesion develops, the non-specific changes of minimal, acute G.T.N. are found in the kidneys.

3. A close hepato-renal correlation is present between 6 and 9 hours, tapering off at 24 hours and absent thereafter.

4. The early changes in the liver and the kidneys bear a considerable degree of similarity to the early changes in these organs under carbon tetrachloride intoxication.

5. Degenerative changes in the liver cord cell consist of swelling which persists for at least 9 hours, mitochondrial clumping and hydropic degeneration. The latter changes disappear after 48 hours. (The hydropic change looks not unlike a cytoplasmic inclusion-body). The liver appears normal by 48 hours, with the exception of scattered binucleate and "dark cells".

6. Degenerative changes in the kidneys are non-specific at first, but by 20 hours they become a generalized and severe coagulative necrosis with extensive and complete sloughing of the necrotic tubular epithelium and disruption of portions of the tubular basement membrane. Subsequently, extensive mitosis and re-epithelialization with cuboidal cells occurs. A late manifestation is the appearance of calcium salts in the loops of Henle and distal portions of the nephron.

7. An acute arteritis occurs between 24 and 48 hours in both large and small vessels.

Experiment 3: Studies on pituitrin toxicity.

(a) Pathological effects of posterior-pituitary extract in the normal rat.

Procedure. Thirty-four rats were segregated for a 3-day period and given the ethanol-anticoagulant mixture plus reduced food intake. Of these, 8 were injected with 0.5 i.u. of an extract of the posterior lobe of the pituitary gland (Parke-Davis "Pituitrin", 10 i.u. /cc), 18 received 0.1 i.u. of the drug and the remaining 8 were retained as normal controls. Animals were then sampled from each group at various intervals and treated as described under general methods.

Results. Time-incidence-severity data are detailed in Table 9, following the description of the lesions in Experiment 3(c).

Morbid anatomy and histology.

Macroscopic. The liver appears normal. The kidneys are markedly congested on their capsular surfaces, with dark venous blood distending the renal veins. These kidneys are very difficult to flush clean with saline and much blood usually remains in the venous (and to a lesser extent in the arterial) channels following the injection of bismuth. The congestion appears as early as 20 minutes and lasts for 4 hours following the administration of pituitary extract.

Microscopic. The liver shows no histological abnormality at any interval and injects well with bismuth.

In the kidneys, the medullary vessels are congested at the earliest interval. By 4 hours there is noted minimal, acute G.T.N. with the large (0.5 i.u.) dose of pituitrin. The acute, scattered, focal cyto-nuclear changes with sloughing are well illustrated in fig. 79. Similar lesions are seen at this dosage in the 6 and 24 hour specimens. No trace of renal damage develops at the low (0.1 i.u.) dosage level throughout the 24 hour period of study.

(b) Pathological effects of pituitrin in subacute liver damage.

Procedure. Thirty-three rats were segregated for 3 days on the reduced food intake plus ethanol-anticoagulant

regimen. They were then given 2 inhalation doses of carbon tetrachloride at 4-day intervals. Five days after the last dose of CCl_4, 16 rats were administered 0.1 i.u. of posterior-pituitary extract (Parke-Davis, "Pituitrin") and the remaining 17 were retained as controls. Varying numbers were employed at subsequent intervals for injection and histological studies as detailed under general methods.

Results. Statistical findings are presented along with the results of Experiment 3 (a & c) in Table 9.

Morbid anatomy and histology.

Macroscopic. The hepatic changes represent those due solely to multiple doses of carbon tetrachloride followed by a 5-day rest (see Experiment 1 (c)). The kidneys are very congested in the animals given pituitrin, from ½ to 6 hours, the hyperemia lessening after this period.

Microscopic. The liver reveals subacute centrolobular necrosis, identical to the features seen between 5 and 7 days in Experiment 1 (c), in both experimental and control alike.

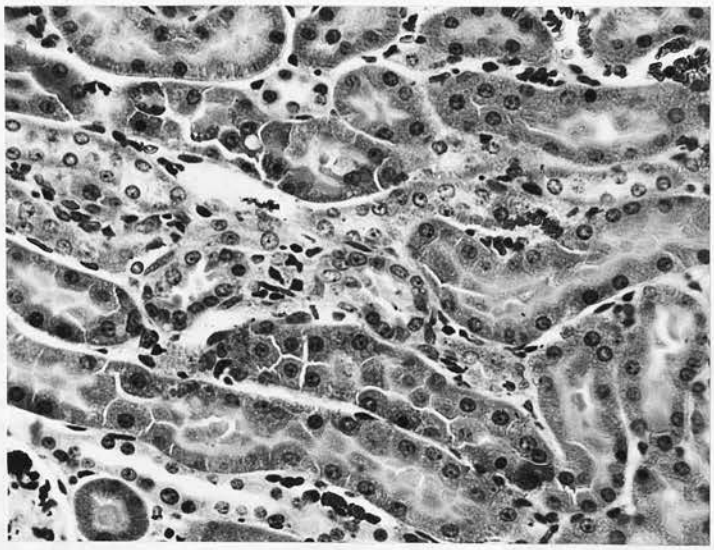


Fig.79. No. B-26. Rat kidney, H&E x300. 4 hrs after 0.5 i.u. of pituitrin to normal rat. Acute G.T.N. (minimal). There are seen the characteristic cyto-nuclear changes in focal fashion.

In the kidneys there is an early, intense vascular congestion of the medullary zone in the experimental group which received pituitary extract. After 4 hours there appear tiny, focal areas of acute G.T.N. superimposed on the 5-day old remnants of subacute carbon tetrachloride G.T.N. From 6 hours onwards, acute tubular necrosis is present in wide distribution and of sufficient severity to suggest the tubular lesion of "focal renal cortical necrosis" (fig. 80). However, no major forms of renal cortical necrosis are seen in this series of investigations, the glomeruli being spared except for changes resulting from increased permeability of the tuft. Subacute G.T.N. in the

corresponding control kidney is shown in fig. 81., where minor changes in the convoluted tubular epithelia and rare cellular casts are all that remain of the earlier lesion.

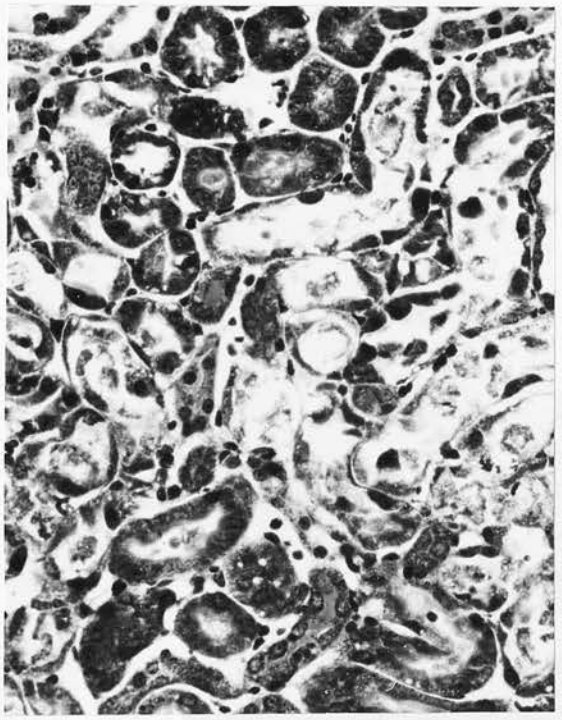


Fig.80. No.C-21. Rat kidney, Tri.x300. 12 hours after 0.1 i.u. of pituitrin. Extensive and severe acute tubular necrosis following pituitrin in subacute liver damage. Compare with figs. 79 and 81.

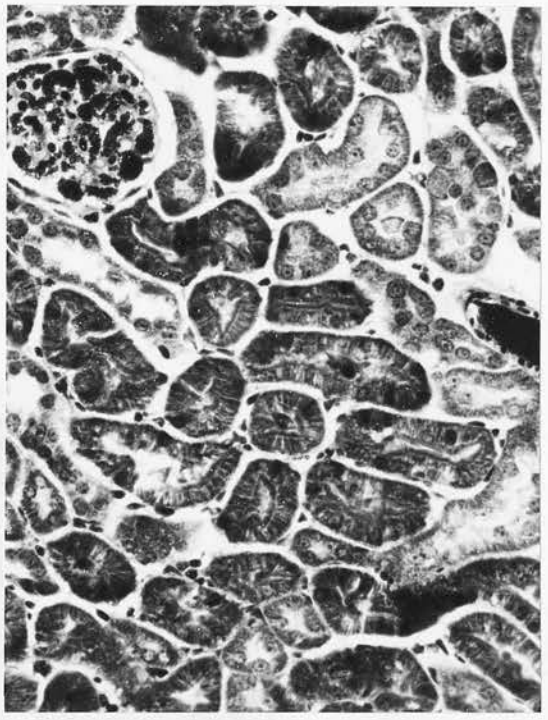


Fig.81. No.C-23. Rat kidney, Tri.x300. Control, 5-day CCl_4 lesion. Mild subacute changes which underlie the changes seen in the previous illustration. Compare with figs. 79 and 80.

The very striking effect of pituitrin in the presence of subacute hepatic necrosis can be most readily appreciated by intercomparison of figs 79, 80 and 81. What is even more impressive is the fact that the lesion in fig. 80 was produced by 1/5th of the dose of pituitary extract

required to produce the lesion illustrated in fig. 79.

The very extensive acute G.T.N. lesion shown in fig. 80 is fully developed within 12 hours and enters a subacute phase between 24 and 48 hours. In this latter phase, casts in the proximal and distal tubules and protein exudate plus some swelling of the epithelium of Bowman's capsule are the outstanding features. Fig. 82 illustrates the subacute phase as described.

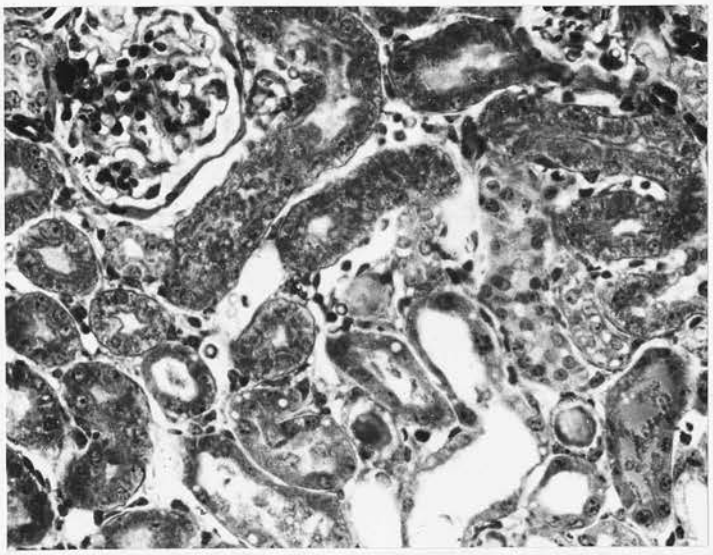


Fig.82. No. C-31. Rat kidney, Tri. x300. 48 hrs after pituitrin, 0.1 i.u., in subacute liver damage of 7 days duration. Note epithelial and protein casts. Also protein exudation in Bowman's capsule and swollen capsular epithelium.

(c) Pathological effects of pituitrin in acute CCl_4 intoxication.

Procedure. Twenty-two rats were prepared for 3 days by reduced food intake and ethanol-anticoagulant mixture, following which they received an inhalation dose of carbon tetrachloride. Twenty-four hours later, 11 rats received 0.1 i.u. of posterior-pituitary extract, the remainder acting as controls. Samples were removed from control and experimental groups at various intervals thereafter and processed according to the procedure outlined under general methods.

Results. Morbid anatomy and histology.

Macroscopic. The hepatic changes are those of acute CCl_4 intoxication of 24 hours duration (see Experiment 1(a)). The kidneys show congestive changes identical to those described in normal animals receiving pituitrin (Experiment 3(a)).

Microscopic. There are no apparent liver lesions other than those induced by carbon tetrachloride.

The kidneys reveal typical acute G.T.N. lesions in an identical degree between control and experimental animals at the 4 hour interval. By 12 hours the pituitrin-injected kidneys display a degree of G.T.N.

far more severe than that seen in the controls (figs.83 and 84 respectively). The involved areas are much more widely distributed and show a far greater impairment of tissues. Numerous protein and cellular casts are found in all types of tubules. There is a considerable degree of dilatation in both the proximal and distal convoluted tubules.

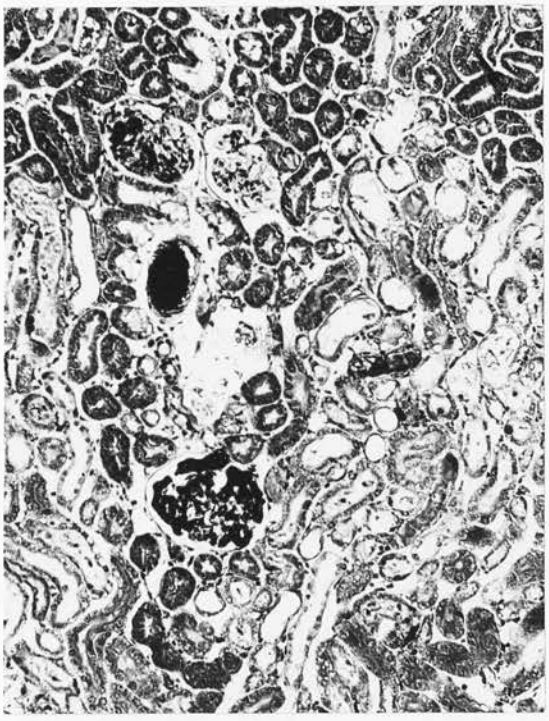


Fig.83. No.C-50. Rat kidney, Tri.x150. 12 hrs after 0.1 i.u. of pituitrin in acute CCl_4 poisoning. Widespread destruction of the convoluted tubules, with dilatation and numerous casts. For comparison with fig. 84.

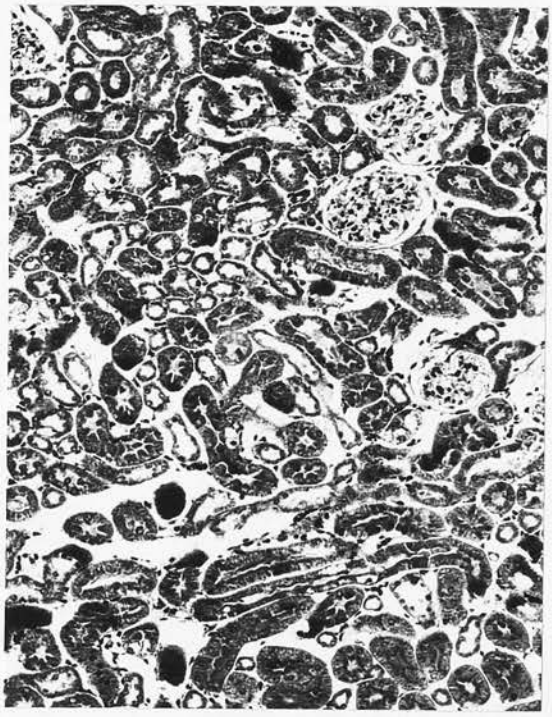


Fig.84. No.C-52. Rat kidney, Tri. x150. Control rat at 36 hrs after CCl_4. Note the characteristic vacuolar necrosis in focal distribution compared to the previous figure.

The picture resembles the pituitrin lesion in chronic liver damage, the acute-on-subacute lesion with carbon tetrachloride

and may even be compared to the 24 hour mercurial lesion in some respects. By 48 hours the pituitrin-treated animal lags behind the control animal in reparative processes, though repair is evident in both cases, signalling a recovery from the toxic action of the drug.

Table 9.

Incidence and Severity of Hepatic and Renal Changes Following Pituitary Extract in Normal Rats and Rats with Liver Damage Incurred by 1 or 2 doses of CCl_4.

	Time after pituitrin.	No. of Rats.	No. with Hepatic Lesion.	Type of[x] Hepatic Lesion.	No. with G.T.N.	Degree of[*] G.T.N.
½ hr interval.						
Normal rats.	½ hrs	1	0	0	0	0
Normal controls.	0	0	-	-	-	-
Subac. hep. nec.	½ hrs	2	2	+,2	2	+++,1
Acute hep. nec.	½ hrs	2	2	++,3	2	++,1
CCl_4 controls Sub.	0	2	2	+,2	2	+++,1
CCl_4 controls Ac.	0	2	2	++,3	2	++,1
1 hr interval.						
Normal rats.	1 hr	6	0	0	0	0
Normal controls.	0	2	0	0	0	0
Subac. hep. nec.	1 hr	2	2	+,2	2	+++,1
Acute hep. nec.	1 hr	2	2	++,3	2	++,1
CCl_4 controls Sub.	0	2	2	+,2	2	+++,1
CCl_4 controls Ac.	0	2	2	++,3	2	++,1

Table 9 contd.

	Time after pituitrin.	No. of Rats.	No. with Hepatic Lesion.	Type of Hepatic Lesion. x	No. with G.T.N.	Degree of G.T.N. *
4 hr interval.						
Normal rats.	4 hrs	5(2 H.D.)	0	0	2(H.D.)	++,1
Normal controls.	0	2	0	0	0	0
Subac. hep. nec.	4 hrs	2	2	+,2	2	++,2
Acute hep. nec.	4 hrs	2	2	++,3	2	++,1
CCl_4 controls Sub.	0	2	2	+,2	2	+++,1
Ac.	0	2	2	++,3	2	++,1
6 hr interval.						
Normal rats.	6 hrs	5(2 H.D.)	0	0	2(H.D.)	++,1
Normal controls.	0	2	0	0	0	0
Subac. hep. nec.	6 hrs	2	2	+,2	2	++,2
Acute hep. nec.	6 hrs	0	-	-	-	-
CCl_4 controls Sub.	0	2	2	+,2	2	+++,1
Ac.	0	0	0	-	-	-
9 hr interval.						
Normal rats.	9 hrs	1	0	0	0	0
Normal controls.	0	0	-	-	-	-
Subac. hep. nec.	9 hrs	2	2	+,2	2	++,3
Acute hep. nec.	9 hrs	0	-	-	-	-
CCl_4 controls Sub.	0	2	2	+,2	2	+++,1
Ac.	0	0	-	-	-	-

Table 9 contd.

	Time after pituitrin.	No. of Rats.	No. with Hepatic Lesion.	Type of Hepatic Lesion. [x]	No. with G.T.N.	Degree of G.T.N. [*]
12 hr interval.						
Normal rats.	12 hrs	2	0	0	0	0
Normal controls.	0	0	-	-	-	-
Subac. hep. nec.	12 hrs	2	2	+,2	2	++,3
Acute hep. nec.	12 hrs	2	2	++,3	2	++,3
CCl_4 controls Sub.	0	2	2	+,2	2	+++,1
CCl_4 controls Ac.	0	2	2	++,3	2	++,1
24 hr interval.						
Normal rats.	24 hrs	2(2 H.D.)	0	0	2(H.D.)	±,1
Normal controls.	0	4	0	0	0	0
Subac. hep. nec.	24 hrs	2	2	+,1	2	++,1
Acute hep. nec.	24 hrs	2	2	++,3	2	++,2
CCl_4 controls Sub.	0	2	2	+,1	2	+++,1
CCl_4 controls Ac.	0	2	2	++,3	2	++,1
48 hr interval.						
Normal rats.	48 hrs	1	0	0	0	0
Normal controls.	0	0	-	-	-	-
Subac. hep. nec.	48 hrs	2	2	±,1	2	+++,3
Acute hep. nec.	48 hrs	1	1	+,2	1	++,1
CCl_4 controls Sub.	0	3	2	±,1	0	0
CCl_4 controls Ac.	0	1	1	+,2	1	++,1

H.D.= High Dose.

[x] Hepatic lesions, average degree: extent:

0	None.	1	Patchy centrolobular
±	Metachromasia.	2	Extensive centrolobular
+	Vacuolar degeneration.	3	Confluent centrolobular
++	Vacuolation plus necrosis		
+++	Necrosis alone.		
++++	Early fibrosis.		

[*] Renal lesions, average degree: extent:

0	None.	1	Minimal, focal.
±	Equivocal G.T.N.	2	Marked, focal.
+	Albuminuria only.	3	Marked, extensive.
++	Acute tubular necrosis		
+++	Subacute G.T.N.		

From table 9 it is apparent that no increased incidence or severity of renal G.T.N. occurs prior to 4 hours after injection with pituitary extract. At this time, normal rats receiving 5 times the standard 0.1 i.u. dose develop minimal, acute G.T.N. and rats with subacute liver damage show the superimposition of the minimal, acute renal lesion on the remains of the 5 day old subacute lesion. There is no increase in the severity of the acute G.T.N. lesion in rats with acute hepatic necrosis at 4 hours. By 6 hours the high-dose normal rats again reveal acute G.T.N., while rats with subacute liver damage show an extension of the acute tubular necrosis to involve widespread focal areas. Control animals with subacute hepatic necrosis remain with a consistent, mild, subacute renal G.T.N. No rats with acute CCl_4 damage were sampled at this interval. No liver damage is sustained from administration of pituitrin at any interval studied. By 12 hours the renal lesion is seen to be very severe in animals with both acute and subacute liver damage plus pituitrin. Their non-injected controls show consistently mild lesions. The same is true of the 24 and 48 hour specimens, but it is apparent that the experimental group with subacute liver damage shows a lesser degree of severity in the renal lesions than does the experimental group with acute liver damage at these intervals.

Summary of the findings in Experiment 3 (a, b & c).

1. A very large dose of pituitary extract (0.5 i.u.) produces the renal lesions characteristic of minimal, acute G.T.N. between 4 and 24 hours after injection into otherwise normal rats.

2. Smaller doses such as employed in the majority of the animals (0.1 i.u. of Pituitrin) fail to produce G.T.N. in normal rats.

3. Posterior pituitary extract, in the amounts employed in this investigation, produces no demonstrable hepatic change.

4. The standard, low (0.1 i.u.) dose of Pituitrin causes a marked increase in the severity of the renal lesions over that of the CCl_4 control animals when given to rats with acute or subacute carbon tetrachloride intoxication. The tubular lesion becomes so severe and widespread as to suggest focal cortical necrosis.

5. The property of greatly increased renal necrotizing activity of pituitary extract when employed in conjunction with carbon tetrachloride is of equal magnitude in both the acute and subacute phases of CCl_4 intoxication, but the necrotizing ability wanes more rapidly in the subacute than in the acute phase.

6. This feature of a more rapid return to normal in the subacute than in the acute phases of carbon tetrachloride intoxication is the reverse to the findings in Experiment 1, Section IV.

7. Untreated normal control rats are uniformly free from hepatic and renal damage.

Discussion

The findings presented in Section IV serve to establish the hepato-renal concept proposed in the previous sections on a very firm foundation. In addition they move a step nearer the solution of the mystery regarding the fundamental pathogenetic mechanisms in operation.

In carbon tetrachloride intoxication, the rat liver lesions after 24 hours are identical to those described by Cameron and Karunaratne in 1936, but the changes noted in the earlier periods of the present series differ from their's considerably. They noted a normal histological appearance at 1 hour, though apparently some mitochondrial changes were present in their 2 hour specimens, while at 5 hours they found only varying degrees of sinusoidal congestion. Glynn and Himsworth, 1948, detected marked swelling of the parenchymal cells from 2 hours onward, with obliter-:ation of the sinusoids and lack of penetration of Indian ink entering via the portal tracts. Both the above groups of investigators administered the drug by subcutaneous injection, which may partially account for the 1 hour delay in the onset of demonstrable hepatic alterations in comparison to the findings in this series. The large vacuolated cells which appear

in this series after 4 hours were shown by Cameron and Karunaratne (1936) to consist of a true hydropic change, the cells giving no reaction for fat, lipoid or glycogen. The mitochondria can be seen pushed to the periphery of the cell and may disappear entirely. With regard to the etiology of the centrolobular necrosis, Glynn and Himsworth (1948) produce well documented evidence in support of ischemia. They injected Indian ink into the splenic vein and demon-:strated it's exclusion from the lobular sinusoids from 2 hours onwards. Similar results have been obtained by Andrews (1948). Loeffler and Nordmann, in 1925, found that under direct observation by transmitted light the intralobular sinusoids were greatly narrowed several hours after administration of chloroform to rats. With a similar technique, utilizing quartz illumination, Wakim and Mann (1942) observed that the sinusoids were practically obliterated 8 hours after exposure to carbon tetrachloride vapour.

It is, then, not a little disturbing to note that Cameron (1950) seriously questions the ischemic etiology of the central necrosis in chlorinated hydrocarbon poisoning. Seneviratne (1949), working with Professor Cameron, studied the sinusoidal blood flow in the rat

liver under direct observation with quartz light trans-illumination. He noted that both chloroform and carbon tetrachloride induce a momentary constriction of the hepatic vessels, followed by persistent dilatation. At no time prior to the onset of necrosis could he discern evidence suggestive of ischemia and the blood flow through the sinusoids was seen to continue very actively until the parenchymal cells became necrotic. His findings are obviously contradictory to those of Himsworth and earlier investigators. They are also incompatible with the changes observed in the present investigation, alterations which will be demonstrated in an even more striking manner in Section V. The bismuth particles, injected via the hepatic artery and the portal vein during the agonal period, are largely excluded from the sinusoids for more than 24 hours, and are seen dammed back in the portal tracts and the peripheral portions of the lobular sinusoids and accentuating these areas, in contrast to the normal liver where bismuth is universal in its distribution and no impedence occurs to normal lobular flow. As Himsworth (1950) points out, the origin of the large, vacuolated, hydropic cells is probably anoxia, since an identical vacuolation occurs when the intralobular circulation is reduced but not entirely arrested (cf. Trowell, 1946).

The surviving outer zone of the lobule varies in thickness in inverse proportion to the dosage of carbon tetra-:chloride (Himsworth, 1950).

Hepatic and renal necrosis have been consistently noted with the chlorinated hydrocarbons in man. Moon (1950) gives an excellent review of the literature on this subject. He notes that 11 of 12 fatal cases presented in his series were alcoholics and he suggests a synergism between ethanol and carbon tetrachloride in both hepato- and nephro-toxicity. The renal lesions consisted of an early hydropic degeneration of the proximal tubules and a late lesion similar to so-called "lower nephron nephrosis".

In experimental animals, on the other hand, the emphasis has been laid on hepatic changes, with surprisingly little reference to the kidneys. A careful search of the literature reveals only one well documented study of the renal injury in rats produced by single doses of carbon tetrachloride (Jennings and Kearns, 1953). These authors employed lethal intraperitoneal doses of the drug in one of their experiments and it is of interest to note that the renal lesion under such circumstances consisted of a widespread necrotizing nephrosis of the cortex, while the livers were stated to be "free of necrosis". Such animals became acutely hypotensive

within half an hour and remained so 'til their death between 2 and 5 hours later, suggesting acute renal anoxia due to shock as the probable etiology of the tubular necrosis. Their 2nd experiment involved the subcutaneous route of administration and the majority of the animals were sampled beyond the 24 hour period. Here they observed no necrosis in the proximal tubules, but found evidence of glomerular permeability to protein plus cloudy swelling of the tubular epithelia. The livers showed the classical centrolobular necrosis.

Earlier workers reported variable findings in a variety of animals. No notable renal lesions with carbon tetrachloride were reported by Gardner et al, (1925) in dogs, Hall and Shillinger (1923) in kittens, Smyth et al (1936) in rats, guinea pigs and monkeys, Mauro (1930) in rabbits and Opie (1950) in rats. On the other hand, Chandler and Chopra (1925) found proximal tubular necrosis in oral carbon tetrachloride poisoning in cats, Gyorgy et al (1946) noted necrotizing cortical nephrosis in rats following chronic inhalation of the drug and Oliver et al (1951) reported a similar lesion in rabbits treated by the subcutaneous route. Reports on chloroform toxicity both in mice (Eschenbrenner and Miller, 1945) and in rats (Opie, 1950, Jennings and Kearns, 1953) have shown consistent renal tubular

necrosis by oral and subcutaneous intoxication.

It is difficult to reconcile the failure of other investigators to produce acute G.T.N. with CCl_4, with the findings of the present investigation. I am in no way convinced that different modes of administration of the drug can explain the presence or absence of renal lesions, shock-inducing methods excluded. Possibly the strain of rat is important, though this again is unlikely. It cannot be explained on the basis of synergism between alcohol and CCl_4 in the present series, for the lesion was produced in the pilot experiments without alcohol. The most probable explanation lies with the staining procedures employed. H&E sections of rat kidneys are very difficult to assess, in comparison with sections stained by Masson's Trichrome. This is particularly so in the very early lesions. The fact that the rat lesions in the present investigation are identical with the early human lesions reported by Moon (1950) is significant. Human kidney tissue is much easier to study, owing to the larger size of each portion of the nephron. Drug dosage may also be a factor.

As mentioned in the introductory remarks, Allen (1951) classes carbon tetrachloride among the many possible etiological agents in cholemic nephrosis. He remarks that the chlorinated hydrocarbons do not

directly affect the kidney. This is likewise the contention of the author. The 5 to 7 hour delay between the onset of the hepatic and the renal lesions suggests that carbon tetrachloride is either non-toxic to the renal epithelia or that its toxicity is much less than it is for the liver cord cell, requiring much longer to exhibit its effects. Histologically there is no apparent comparable degree of swelling in the tubular epithelial cells in the early intervals which might produce an obliteration of the vascular bed such as occurs in the intralobular circulation of the liver. Yet it seems likely, from the results of experiments to be detailed in Section V, that there is, in fact, a partial ischemia of the peritubular capillaries 4 hours after inhalation of carbon tetrachloride. Though it cannot be proven that the ischemia is not of a 'swollen-obstructive' variety, it seems more likely to be due to efferent arteriolar spasm. This is the site which Allen (1951) considers most likely to become atretic in any vasospastic, ischemic process in the kidney (fig. 6, A & B). It could best account for the main histological features of minimal protein-:uria and tiny foci of acute degenerative changes in the tubular epithelia seen in the 6 and 8 hour specimens.

When the probable renal ischemia is considered together with its onset after the liver has been demonstrably ischemic for a few preceding hours, plus the fact that there is a close degree of temporal correlation between the duration of the hepatic and renal changes, it seems very reasonable to suggest that the renal changes are consequent upon liver failure in respect to its capacity to detoxify vaso-active compounds in the circulation. All these changes occur in the absence of cholemia, so the term "bile nephrosis" in inept in this series of studies.

Vacuolar, hydropic degeneration of the proximal tubular epithelium is discussed extensively by Allen (1951), who considers the lesion to represent an osmotic dysadaptation of the epithelial cell. A wide variety of etiologic agents may produce these hydropic changes. The classical examples are those produced by parenteral administration of hypertonic solutions. On the other hand, he points out that the vacuolar changes observed in cholemic nephrosis (see fig. 37), dysentery (Jaffe and Sternberg, 1919) and ulcerative colitis (Jensen et al, 1950, Kulka et al, 1950) are morpho-:logically distinguishable from the osmotic forms due to the coarse, large irregular features. These coarse

features are probably due to the underlying mitochondrial damage in the former group. The swelling usually accompanies a smudgy or hyaline-granule pattern in the cytoplasm of the same cell. It is possible that a causal relationship exists between the earlier mitochondrial change and the later hydropic vacuolation, the altered dispersion of the mitochondrial elements producing osmotic imbalance in the cell. This type of hydropic degeneration is usually irreversible, the cells burst and are sloughed-off in various stages of integrity. The absence of polysaccharide material in the hydropic epithelial cells of both kidney and liver proves that hydropic degeneration in these sites is in no way related to the type seen in pancreatic beta cells, where Toreson (1951) showed the degeneration to be due to glycogen infiltration.

The hepato-renal concept is further supported in the present series by the results obtained with multiple intoxication with CCl_4. The lag period between the hepatic and renal lesions is not appreciably shortened, though the duration of the lesions is definitely protracted and the severity many-times increased with subacute liver and kidney damage. It is worthwhile noting that the 3rd dose of drug was administered at a time when the

renal lesions could be expected to be practically healed and while the liver would still be undergoing active regenerative replacement of its functional substance. The resulting further loss of functioning liver tissue should then be expected to far exceed the anticipated loss of the repleted renal tubular epi-:thelium, whereas, in actuality, the renal lesion increases in severity to a degree quite comparable to that of the liver. This then, constitutes further supportive evidence for the concept of the hepatic pathogenesis of the renal disorders. It is difficult to imagine any increase occurring in the toxicity of CCl_4 to the histologically regenerated tubular epithelia, were this toxicity expended directly on the cells in question.

What has been said of the concentration of pathological lore on the liver to the practical exclusion of the kidneys in carbon tetrachloride intoxication is found to apply in reverse to mercury poisoning. Whereas several well-documented treatises exist on mercurial nephrosis (cf. Edwards, 1942, Allen, 1951, etc.), references to the hepatic changes in the literature are few and far between, while standard text-books of pathology ignore the hepatic changes completely.

Harmon (1928) discussed the liver lesions in 4 human autopsy cases, while Ogilvie (1932) reported two human autopsies and the observations on 16 experimental rabbits, stressing the hepatic changes. Both authors noted mild to moderate hepatic congestion, with cloudy swelling of the cord cells. In the rabbits and humans alike, the cells near the centres of the lobules developed a characteristic vacuolar hydropic degenera-:tion and clumping of the mitochondrial granules. These changes are in complete agreement with the changes observed in the rat livers in this present series. Excessive mitosis, such as reported in human livers by Pilliet and Cathineau (1892), Heitzmann (1918) and McNider (1924) (quoted in Ogilvie, 1932), is not apparent in the rat. One further finding of interest emerged from Ogilvie's experimental investigations, namely an excessive increase in the blood lipid content, which was noted to practically double its concentration within 3 to 4 hours after administration of the $HgCl_2$, then to fall and subsequently become re-elevated. This rise in blood lipid was not associated with any deposition of lipid in the liver cord cells such as seen in phosphorus or phloridzin poisoning, nor was there any correlation between the hyperlipemia and the blood alkali reserve (CO_2 combining power) (Ogilvie, 1934).

Ogilvie's results have been discussed in some detail in that they bear considerably on the findings of the present series. It will be recalled that within 1 hour, the liver cord cells are swollen and the sinusoids practically obliterated, while 9 hours later the swelling appears to be starting to subside. Hydropic degeneration occurs in cells near the centre of the lobule and mitochondrial clumping is seen, evidence pointing to centrolobular ischemia and not too unlike the CCl_4 effect. The very early hyperlipemia would suggest that there is a very rapidly developed metabolic dysfunction in the liver. When the early renal changes of non-specific G.T.N. as seen 6 and 9 hours after administration of $HgCl_2$ are now considered in the light of the hepatic changes, it is obvious that we are again dealing with a beautiful example of hepato-renal correlation, strikingly similar to the findings in carbon tetrachloride intoxication.

It could be argued that the mercuric chloride damage in the kidney is, from its incipience, a mercury-specific nephrotoxic nephrosis. Oliver et al, (1951), do not believe this. They claim, from studies of individual dissected nephrons, that the mercury lesion is of dual origin, the tubulorrhexis resulting

from renal ischemia and the massive necrosis due to the specific nephrotoxicity of the mercuric ion. As Edwards (1942) points out, mercuric salts are most toxic to the first portions of the proximal convoluted tubules since the poison strikes this epithelium in maximum concentration. As stated in the findings of Experiment 2, the rat renal lesion in $HgCl_2$ intoxication would appear to be definitely of biphasic nature. The mild, focal and non-specific type of G.T.N. which first occurs is replaced between 9 and 20 hours by an extreme coagulative necrosis which wipes-out most of the proximal, and, to a lesser extent, distal convoluted tubules of the cortex. This is in striking contrast to CCl_4 G.T.N., which is always a focal lesion. The tendency of approximately 50% of the rats in this series to develop a cortico-medullary distribution of the lesion is in keeping with the experimental findings of Wainright, 1950, but is contrary to Allen's (1951) observation that in human mercurial nephrosis the lesion is distinctly cortical in distribution. The dose is very possibly the deciding factor. Wainright's dosage in rats was double that used in this series, but probably far less than the human intake. Both the above authors suggest that the distribution of their respective lesions indicates a circulatory factor in the pathogenesis of mercurial nephrosis.

The nephrotoxicity of mercuric salts is presumed to result from the interaction of the mercuric ion with the sulfhydryl groups of the cells. BAL supposedly protects against mercuric salts by successfully competing with the sulfhydryl groups for the mercury (Waters and Stock, 1945). The mercuric-sulfhydryl compound must occur in the liver, causing the immediate hepatic cord swelling. And yet necrosis is extremely uncommon in the liver and is centrolobular in distribution when it is seen, implicating anoxic rather than toxic necrosis. If this reasoning be applied to the renal lesion, the circulatory factor will be seen to be of far greater importance than the toxic factor in the genesis of mercurial nephrosis.

The possibility that mercury might produce an early, acute swelling of the tubular epithelium, with purely mechanical obliteration of the peritubular capillaries, cannot be excluded. There is, however, no histological proof that this does occur. Again, it is not inconceivable that the mercurial action is directly vasospastic to the renal efferent arterioles, the hepatic changes being a chance association and not pathogenetically related. This is also unlikely when the time lag between the appearance of the liver and kidney lesions is considered.

When all the available evidence concerning the mercurial lesions in the liver and kidneys is weighed and assessed, the conclusion that we again deal with an hepato-renal manifestation seems highly logical. That the intralobular circulation of the liver is seriously impeded will be demonstrated conclusively in Section V. That this impedence of blood flow produces ischemia and immediate hepatic dysfunction is apparent from the hyperlipemia within 3½ hours, as shown by Ogilvie (1932, 1934) and by the subsequent development of centrolobular hydropic degeneration, a characteristic of severe hypoxia. The presumed sequence of events would then follow an identical pattern to that postulated for the chlorinated hydrocarbons with hepatic failure in regard to the detoxification of circulating vaso-active materials and consequent arteriolospasm in the kidneys. Superadded upon this sequence there develops between 9 and 20 hours a specific nephrotoxic necrosis of the already ischemic tubules. With the appearance of this more severe lesion, the purely ischemic lesion is obliterated.

The most interesting and significant findings in Section IV have resulted from experiments involving the use of posterior pituitary extract, both in normal

animals and in animals with acute or subacute hepatic necrosis. Here a decided advance has been made in our understanding of the pathogenetic relationship between the liver and kidneys. Since the observations of Fauvet in 1931, it has been known that very large doses of pituitrin will produce experimental focal necrosis in the liver and kidneys. This was confirmed in 1937 by Végh and von Pallos and more recently by Govan and Mukherjee (1950, b). The latter group of investigators used dosages of the order of 10 i.u./2kg. in rabbits, daily, equivalent to between 0.5 and 0.6 i.u. /100 gm rat / day. This dosage is 5 or 6 times the standard single dosage of 0.1 i.u. employed in the present investigation. It is of interest to note that after a week or more their hepatic changes were primarily those of hydropic degeneration, while the renal lesions were very similar to the findings of the present series when normal rats received a single large (0.5 i.u.) injection of pituitrin.

In the recorded observations of the present experiments, one observes a very striking renal necrogenicity of 0.1 i.u. of pituitrin when used in conjunction with acute or subacute carbon tetrachloride liver damage.

This must be compared with the absence of lesions in the kidneys of normal rats receiving the same dosage, and with the relatively mild renal lesions in the carbon tetrachloride controls. The question then arises, - does this represent a synergism between carbon tetrachloride and pituitrin with regard to their respective individual ability to produce minimal, acute G.T.N.? Two features of combined pituitrin-tetra-:chloride toxicity make this suggestion highly unlikely.

1. The necrogenic property of 0.1 i.u. of pituitrin is of exactly the same order of magnitude in both the acute and the subacute phases of CCl_4 intoxication. Were synergism responsible, this would not be so.

2. This same necrotizing effect is seen to disappear more rapidly in the subacute than in the acute phase of CCl_4 intoxication, the reverse to the findings in single versus multiple-dose carbon tetrachloride lesions. Again, synergism would be expected to exaggerate the underlying tetrachloride effects, not reverse them. We have thus excluded synergism as a likelihood, while we know that 0.1 i.u. of pituitrin is completely non-toxic to the renal tubules of the normal rat. Earlier in the discussion it was pointed out that the carbon tetrachloride effect was very unlikely to be expended directly upon the tubular epithelium.

We are thus led to the conclusion that the action of pituitrin is indirect upon the kidneys. To be sure, the lesion which is produced is very non-specific. It is identical to acute G.T.N. (of varying grades of severity) as seen in Section II in human kidneys from a wide selection of disease entities, in Section III with both natural disease and surgically-induced ischemia of the rabbit liver, and in Section IV with the so-called "toxic nephroses" of carbon tetrachloride and early mercury poisoning in rats. In multiple-dose pituitrin studies in the rat (Vegh and von Pallos, 1937; Govan and Mukherjee, 1950), hydropic degeneration of the proximal convoluted tubular epithelium has been described. Such changes have been recorded in human kidneys where associated hepatic damage has been present, (Kulka et al, 1950; Allen, 1951; fig. 37, Section II, this thesis) and also in the rat kidneys from Experiments 1 and 3, Section IV. The lack of specificity of this vacuolar change suggests a non-toxic etiology. What could be more reasonable than to suggest that it occurs as a result of anoxia consequent upon vasospasm? It would thus be closely analogous to the hydropic degeneration seen in the hepatic cord cells as a consequence of anoxia (Trowell, 1946; Himsworth, 1950) and of repeated injections of pituitrin (again undoubtedly anoxic) (Govan and Mukherjee, 1950, b).

The above line of reasoning establishes the fact that the renal lesion under discussion (G.T.N., acute tubular necrosis, "ischemuric" nephrosis, etc.) is of disturbed vasomotor (ischemic) pathogenesis. This fact is now widely accepted (cf. Scriver and Oertel, 1930; Campbell and Henderson, 1949; Oliver, 1951; Allen, 1951; Sheehan and Moore, 1952; Bull and Dible, 1953). Infinitely more important, as judged from the recorded observations of this study, is the constant interpenetration of hepatic damage into this ischemic renal cycle. It is most gratifying to have demonstrated that a hormonal vasospastic substance, present at all times in the circulating blood and normally detoxified by the liver, can have its necrogenic activity so markedly potentiated by liver damage. It allows the formulation of a reasonable pathogenetic hypothesis to explain the very consistent hepato-renal correlation which has been observed throughout these studies and substitutes the word "probable" for "possible" in discussions of the findings of the earlier sections.

The following is the likely pathogenetic inter-:relationship between combined liver and kidney damage:

1. The liver is a key in the normal homeostatic mechanism responsible for regulation of the circulation in that it inactivates the vaso-excitatory hormone, pituitrin, maintaining a constant blood level of this substance.

2. Liver damage, when of sufficient degree, results in failure of the organ to detoxify pituitrin, allowing a rise in the titre of this vasospastic material in the peripheral circulation.

3. Excessive amounts of pituitrin in the circulation bring about arterial spasm throughout the body, the effects being most felt in organs which, through some peculiarity of their vasculature, are hypersusceptible to vasospasm and become anoxic. The sites in which such lesions most commonly occur are 1, the kidney, and 2, the placental site.

This interpretation will include all other vaso-:active materials which are normally inactivated by the liver, if such exist. It has only been proven to apply to posterior pituitary hormone(s).

It is questionable as to where the V.D.M. - V.E.M. hypothesis of Shorr, Zweifach, Furchgott and Baez (1947) enters this possibly over-simplified system, if at all. They described the existence of a homeostatic system for the regulation of the peripheral circulation as follows: "This system is made up of two components, one of hepatic (V.D.M.), the other of renal (V.E.M.) origin. Evidence of the presence of such a circulatory homeostatic system, its mode and sites of origin, and the manner in which it regulates the behaviour of the peripheral vascular bed, was first derived from studies on experimental hemorrhagic and traumatic shock. Subsequent studies have shown this homeostatic system to be likewise involved in the syndrome of renal

hypertension". They pointed out that the most probable mechanism responsible for the formation of these vasotropic factors during the shock syndrome is the reduced oxygen tension which results from the reduction of blood flow to the liver and skeletal muscle (V.D.M.) and the kidney (V.E.M.). In my own experience, irreversible forms of shock commonly reveal, at autopsy, focal necrosis of the liver, usually of centro-:lobular distribution, while the kidneys present the features of acute or subacute tubular necrosis. This is well illustrated in the human case presented in Section V, and is confirmed by Moon (1948). In other words, shock may precipitate the hepato-renal syndrome and invoke the sequence which I have just described. The question must be considered open.

Returning to the subject of the severity of the pituitrin lesion in rats with liver damage, it must be admitted that a full-blown renal cortical necrosis did not develop. On the other hand, the severity and distribution of the tubular damage was equal to the tubular component of "focal renal cortical necrosis". Sheehan and Moore (1952) presented a graded series of renal lesions from simple albuminuria and epithelial

casts up to extensive tubular necrosis which they observed in 26 out of 67 autopsied cases of utero-placental apoplexy. The majority of the remainder showed the more extensive lesions of renal cortical necrosis. They considered these cases to represent increasing degrees of severity up to true cortical necrosis, of renal lesions with a single etiology, namely vasospasm, the type of lesion depending on both the site and the duration of the spasm. The etiology of the spasm was unknown. The vast majority of cases of renal cortical necrosis are found in eclamptic toxemia of pregnancy (Duff and More, 1941). Govan and Mukherjee (1950, a,) studied the variable liver lesions in human eclampsia and found the commonest lesions to be fibrinoid centrolobular or midzonal necrosis. Three of the 20 cases studied showed periportal necrosis (the so-called "typical" lesion in the older literature), but in each of these there was definite thrombosis of the portal vein; while 5 of the cases presented a severe, foamy vacuo-:lation of the cord cells without actual necrosis.

Since renal cortical necrosis and utero-placental apoplexy are all too frequent accompaniments of eclamptic toxemia, I have placed the utero-placental

junction as the 2nd most common site of the untoward activity of the undenatured vaso-excitatory substance in liver damage. The spiral construction of the endo-:metrial arterioles makes them unduly liable to complete occlusion in spastic conditions, as held by the most widely accepted view on the mechanism of menstruation. It would seem reasonable to assume that the same ischemic episode precedes both the renal and the uteroplacental changes in renal cortical necrosis plus uteroplacental haemorrhage associated with eclamptic or pre-eclamptic toxemia of pregnancy. Dieckmann and Michel (1937) have demonstrated an increased sensitivity of toxemic patients to pituitrin. They suggest that if the syndrome is related to pituitrin it must be due to either (a) over-secretion, (b) decreased neutraliza-:tion, or (c) an increased susceptibility to its action. Govan and Mukherjee (1950, b) favour the 3rd explanation since they claim to have demonstrated a synergistic action between the anterior and posterior pituitary hormones. If such be the case, every pregnancy should develop eclampsia. I suggest that this does not occur because the actual control lies with the liver. However, the status of the liver early in the course of toxemia of pregnancy remains unknown, which leaves a large hiatus in this purely theoretical side-line to the more pertinent aspects of the hepato-renal syndrome as studied in the present investigation.

Summary

1. Glomerulotubular nephrosis has been produced by the administration of CCl_4, $HgCl_2$ and pituitrin in single doses to rats.

2. Carbon tetrachloride produces immediate swelling of hepatic cord cells, with obstruction of the intralobular circulation.

3. Within 5 hours after the hemodynamic alterations in the liver, the earliest acute, focal lesions appear in the kidneys. These are primarily a slight proteinuria and the initiation of acute tubular necrosis in proximal and distal portions of the nephron.

4. The fully developed G.T.N. lesion shows a focal, marked, vacuolar hydropic degeneration of the proximal tubular epithelial cells such as seen in human cases with extensive liver damage. These vacuoles do not contain polysaccharide material, as shown by the P.A.S. stain.

5. The correlated hepatic and renal changes are considered a manifestation of the hepato-renal syndrome (see text).

6. Mercuric chloride produces a similar hemodynamic alteration in the liver within 1 hour of administration.

7. The early mercurial renal changes between 6 and 9 hours are identical to the changes in CCl_4 intoxication, namely acute, minimal G.T.N. These changes are obliterated between 9 and 20 hours by the onset of widespread coagulative tubular necrosis throughout the cortical tissues.

8. The renal lesion in mercury poisoning is considered to be biphasic in nature, an early ischemic lesion and a superadded nephrotoxic necrosis. The initial ischemic phase is considered to represent an hepato-renal manifestation rather than a direct ischemic action of mercury on the kidneys, considering the 5 hour delay between the onset of the two phenomena at the histo-logical level and the close similarity to the pattern of the carbon tetrachloride changes.

9. The toxic and ischemic properties of mercuric salts are discussed in relation to the chemical principles involved and the respective findings in the two organs.

10. An acute, smudgy, fibrinoid degeneration of the medial coats of arterial walls is present between 1 and 2 days after mercurialization.

11. The occurrence of minimal, acute G.T.N. in normal rats by way of a single massive dose of posterior pituitary extract has been confirmed.

12. One fifth of the dosage of pituitrin required for the production of the minimal lesion in normal rats will produce a very severe focal G.T.N. lesion in rats with acute or subacute liver damage of carbon tetrachloride etiology.

13. Reasons are put forward to exclude both synergism and direct nephrotoxicity of pituitrin and CCl_4.

14. The following hypothesis is presented, based on excellent supportive evidence. (1) That the liver is a key in a homeostatic, humoral system for the regulation of the circulation. (2) That the failure of a damaged liver to denature the steadily-produced vasospastic materials such as pituitrin, allows a rise in the titre of such substances in the circulating blood. (3) That such compounds produce widespread vasospastic changes throughout the vascular tree in the body, but these are most felt in organs whose vascular architecture predisposes to acute anoxia of the vascular area of supply under conditions of spasm. The commonest, though by no means the only, such sites are the kidneys and the uteroplacental junction.

15. The significance of this hypothesis is discussed in relation to eclampsia, with particular attention to the features of liver damage, uteroplacental apoplexy and renal cortical necrosis.

SECTION V

SECTION V

Arteriographic studies in the pathogenesis of correlated hepatic and renal lesions in the rat.

The previous sections have demonstrated a convincing correlation in several mammalian species between a variety of hepatic lesions, and a non-specific renal lesion which has been termed glomerulo-tubular nephrosis. In addition, evidence has been presented favouring an ischemic etiology for this non-specific renal lesion. The general acceptance of the role of vasospasm in acute degenerative lesions of the kidney has been outlined by several workers (c.f. Scriver and Oertel, 1930: Campbell and Henderson, 1949: French, 1950: Oliver, et al, 1951: Allen, 1951: Sheehan and Moore, 1952: and Bull and Dible, 1953; etc.), yet direct microarteriographic proof of the existence of this phenomenon is lacking. In the past, in-vivo attempts have been made to demonstrate arteriospasm and/or arterio-venous shunt mechanisms in conditions of shock, (Trueta et al, 1947) and in corrosive sublimate poisoning (Wainright, 1950). Unavoidable or intentional shock in the above investigations imposes very stringent restrictions upon the interpretation of the results, since both groups of investigators depended upon the propulsive action of the animal's heart for the conveyance of their

injection media, and, as Oliver (1951) points out, the results can most readily be explained on the basis of a failing circulation. Herdman and Jaco, 1950, reduced the renal blood flow unilaterally in the rabbit by means of a clamp and found that the circulation was better maintained in the deep than in the superficial cortex, as shown with intra-vascular dyestuffs. Such results, reputed to demonstrate the Trueta shunt, actually serve to further strengthen Oliver's simple and adequate explanation that when blood enters an organ under reduced pressure, the most distal parts will receive blood latest and least.

In the present investigation, since vasospasm was so strongly implicated, it seemed desireable to attempt to show its existence in the serial-time studies under-:taken in Section IV, and to attempt to correlate the histological lesion at any given interval with the state of the vascular tree at that time. The experimental demonstration of arteriospasm in the living rat would have necessitated excessive expenditures on costly apparatus and contrast media, and would have involved complex and unreliable techniques. It seemed possible that if the radio-opaque injection mass were introduced during the agonal phase, the blood-vessels might retain their pre-existing vaso-motor tone for a sufficient period of time to allow fixation of the organ and its

vascular tree prior to radiography. This raised two important questions; 1. does spasm, if present, persist for a sufficient length of time to allow of any known method of fixation? and 2. what effect does the agonal state produce in the blood-vessels?. Though no known answer existed, it seemed desireable to investigate the problem, employing as the only possible defence an adequate amount of control material.

The term "microradiography" was fashioned by Pierre Goby in 1913; defined as the use of the roentgen ray in the visualization of microscopic detail. He utilized x-rays for the study of skeletal details in crustaceans, obtaining excellent enlargements (up to 25 times) from fine-grain photographic plates. This aspect of radiology had, in itself, a very restricted degree of utility. Lamarque (1936) applied the technique to the demonstration of cellular detail in soft tissues and suggested that the science be named "historadio-:graphy". Microarteriography, a very fruitful subdivision of microradiography, was first employed by Grechishkin and Prives in Leningrad , 1935, using thorotrast as a contrast medium. Bohatyrtschuk, in 1944, working in Kiew, developed microarteriography, or as he termed it, microvasoroentgenography, to a very fine art, and Barclay used very similar techniques at Oxford

between 1947 and 1949. The magnificent results which he obtained were published posthumously in 1951. These later techniques utilized extremely fine-grain photo-:graphic emulsions, necessitating soft (Grenz) x-rays, with long exposure periods for adequate contrast. A recent monograph by Bellman (1953) gives a complete survey of the literature and a thorough discussion of modern microangiographic methods.

In the present work, the earliest results obtained with contrast media in rat kidneys were interpreted as showing changes suggesting spasm in the larger arteries under the influence of carbon tetrachloride. A great deal of time and material was subsequently expended in an attempt to obtain consistent changes of this kind. After considerably more experience, it became obvious that these early leads were erroneous and due to intra-vascular clotting and various other causes of inadequate filling. Much of the microarteriographic approach as employed in the present investigation was new and untried. I have labelled the ensuing heading "Investigations into methods", but it should be borne in mind that minor alterations in technique were added throughout the investigational period and further alterations are contem-:plated at the time of writing. Much of the work entailed will require repetition, the findings having

provided a solid foundation for further studies.

Investigations into methods.

An injection apparatus was constructed along the lines of that shown in fig. 85. It consisted of a large air reservoir inflated by a valved hand-bulb, a mercury manometer, an injection chamber and a saline-flush bottle.

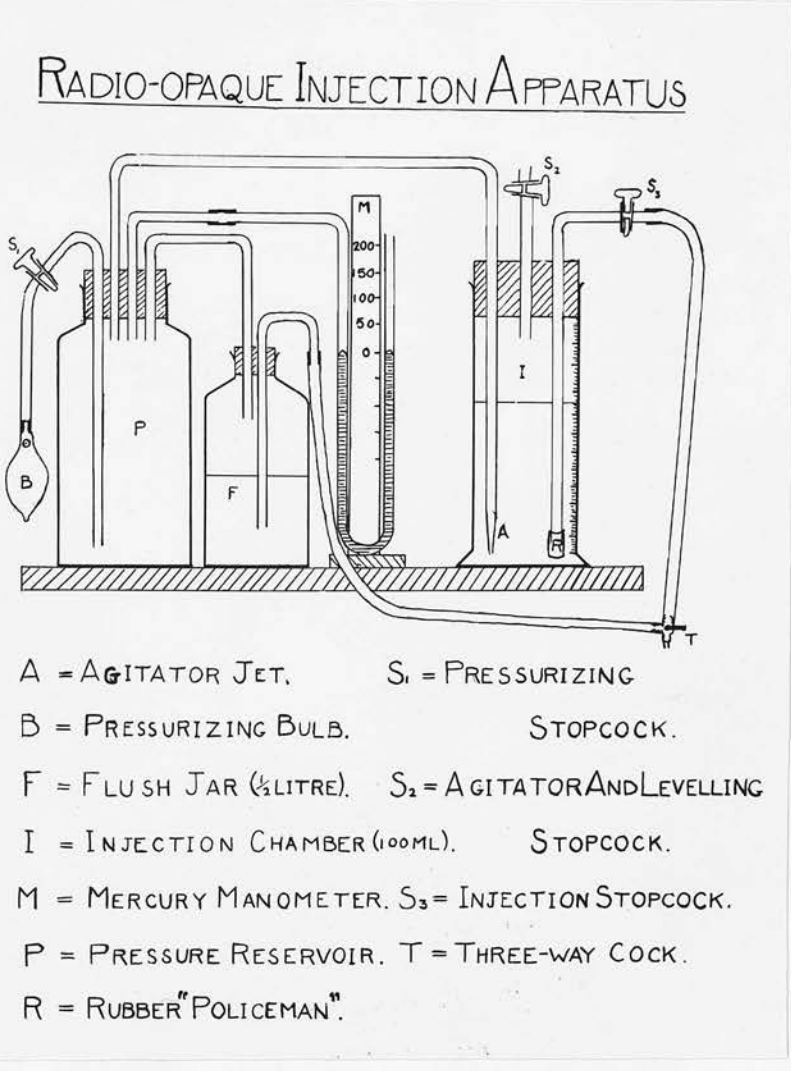


Fig.85. The injection apparatus.

The leads from the injection and flush chambers were joined by a three-way metal stop-cock which accepted a large-bore 'Record' needle on its distal aspect. The needle was inserted into one end of a 12 inch length of 51 gauge polythene tubing, the other end of which was drawn out to give a cannula tip small enough to slip easily into the aorta of a 100 gram rat. An air-escape valve was fitted to the injection chamber for the purposes of adjusting the injection pressure and allowing air-agitation of the solid suspension between injections, the air entering from a narrow nozzle close to the bottom of the injection chamber.

A wide variety of contrast media has been employed in the past for arteriography. For "in-vivo" experiments, thorium dioxide (thorotrast) is most suitable due to its extremely fine particle size (about 0.1 microns) and relatively slight toxicity. Unfortunately its cost is prohibitive for large scale investigations. A large variety of iodinated hydrocarbons are suitable for rapid arteriographic studies, but, being of molecular size they are soluble and diffusible, leaving the vessels for the tissue fluids. They are also somewhat inferior in their radio-opaque properties compared to the metallic salts. For arteriography in dead or dying tissues, insoluble metallic salts are the ones

most commonly employed. Most of these have particle sizes between 6 and 12 microns, vermillion being at the lower and bismuth oxychloride at the upper end of the range, and they are exceptionally radio-opaque. Examples of such are red oxide of lead (red lead), colloidal silver iodide, vermillion (mercuric sulfide) and bismuth oxychloride. All have the dis-:advantage of agglomerating on standing, but are usually easily re-suspended by agitation in a high-speed tissue macerator. This objection applies most seriously to bismuth oxychloride. There is one very important distinction between the contrast media and their respective uses in living and in dead tissues. Media injected into the blood stream of the living animal usually effect only a partial replacement of the vascular stream with the opaque matter, those inserted into dead tissues replace the blood entirely with contrast medium.

Various contrast media were tested initially. These included 10% silver iodide; 10% freshly precipitated (yellow) mercury sulfide; 10, 20 and 30% bismuth oxychloride; 20% vermillion (mercuric sulfide): and 20% red oxide of lead; all suspended in 10% plasma protein and tap water. Indifferent results were

obtained with the 10% suspensions due to relatively poor contrast. The 20 and 30% metallic salts gave excellent contrast, and it was decided that 30% bismuth oxychloride gave the best contrast of all media. The 20% vermillion gave brilliant radio-opacity and clearly defined the glomerular tufts. It was disregarded due to its proclivity for filling the capillary beds, resulting in excessive background shadow.

It was found that failure to fill the arterial tree of the kidney occurred frequently, and preliminary flushing with heparinized saline was instituted to minimize intravascular clotting. This was aided by the addition of phenylindanedione to the rat drinking water three days prior to use, and later by the simple expedient of nicking the left renal vein to encourage drainage by the elimination of back-pressure.

Preparation of the rat for injection was inevitably a highly traumatic procedure, with a strong likelihood that neurigenic spasm would invariably accompany the method in spite of the very deep anesthetic range obtained with ether. As a result, it was decided to employ a ganglion-blocking agent in the late pre-agonal phase. Of the many such agents tested (see preliminary experiments of Section IV), hexamethonium bromide was

selected and employed throughout at a dosage of 10 mgs / rat, intramuscularly. Consequent upon the use of anticoagulants, saline, hexamethonium bromide and the practice of nicking the renal vein, it was possible to obtain good filling of the renal vascular bed in 90% of animals. In the 10% of failures, the contrast medium usually filled most of the major branches and their subdivisions and left a clearly defined filling defect in the occluded branch(es) as an obvious artefact. Such failures of injection were evident at the time of injection since the kidney(s) would not "clear" properly with perfusion and the uncleared areas would not take on the colour of the contrast medium.

Choice of an appropriate x-ray film was not difficult. The requisite features were that it have a grain size of a sufficient degree of fineness such that it could be enlarged to approximately 10 times, and that it have a sufficient emulsion speed that it be adequately exposed in short exposure periods by the hard x-rays emitted from routine diagnostic x-ray apparatus. Two such films were readily available on the market. Ilford Contrast Dental Film, 15P was the first to be used. It gave excellent contrast and enlarged to between 8 and 10 times without too objectionable grain interference. This film came

supplied only in small dental cassettes, ideal for the radiography of single rat kidneys. It had the dis-:advantage that only a few kidneys could thus be exposed at any given time, and since the automatic timer on the Watson clinical x-ray unit varied slightly at each exposure, a degree of contrast variation resulted which proved very distracting to the photographic unit which enlarged the films. An added disadvantage was encountered when it was decided to include the liver in the arteriographic studies, the rat liver being slightly too large for the dental film. As a consequence, Kodak Crystallex became the film of choice. This had an even finer grain size than the Ilford film and was supplied in a wide variety of packagings. The 8 by 10 inch size was found most suitable for routine use.

The effect of kidney posture in relation to the x-ray film and beam was investigated and found not to be significant. In addition, the influence of fixation on the vascular diameters was studied in both normal and assumed vasospastic states (4-hour carbon tetra-:chloride intoxication). After 24 hours formalin-fixation there was a very slight (less than 10%) reduction in the size of measurable vessels as viewed at 6 magnifications, in comparison to the vascular diameters in quick-frozen kidneys. This change was apparent in both normal and experimental kidneys.

On the other hand, definition and contrast were far superior in the formalin-fixed kidneys, and the preparation of adequate tissue slices required more than frozen-fixation in order to immobilize the bismuth medium.

To analyse and assess the arterial tree in the normal and various abnormal conditions (carbon tetra-:chloride, mercuric chloride and pituitrin nephroses), the contact x-ray film was examined by a variety of methods of magnification. These included observation under the scanning lens of the standard microscope (X30) which proved undesireable due to grain inter-:ference; observation under the low-power stereoscopic dissecting microscope, giving approximate magnifications of 6 and 12 times, which had the drawbacks that the films had to be viewed individually and could not be assessed from the viewpoint of actual luminal diameters of the larger blood-vessels; and finally by photographic enlargements. This latter made comparison easy and allowed direct vascular measurements to be obtained by calipers from the print. With a very few exceptions, the full-thickness x-ray of the kidney was found to be uninformative. The observations on the various forms of ischemic nephrosis detailed in Section IV demonstrated

conclusively that no spasm could be identified in vessels larger than the interlobular arteries (.i.e. in the arcuate and interlobar arteries). Nor could significant changes be interpreted from the condition of the interlobular arteries, afferent arterioles or glomerular tufts, due, in good measure, to the crowded image obtained.

Therefore it was decided to attempt the radiography of thin longitudinal sections of the kidneys. This brought in errors due to the location of the kidney slice relative to the anterior and posterior surfaces and to variations in distance from the hilar plane. For purposes of contrast, the thicker the section the better; for purposes of detail, the thinner the better. Initially the sections were cut at 1500 microns, but it was found that with well injected kidneys, 700 microns would give good contrast. From the x-rays of such sections, enlarged to 10 magnifications, it appeared possible that spasm was present in arteries of inter-:lobular dimensions and below in both CCl_4 and $HgCl_2$ intoxication. However, the limits of magnification were reached at 10 times, both from the point of view of grain interference from the film emulsion and of the low resolving-power of the photographic paper.

In addition, an excessive amount of structural detail was present on the film, masking the more minute changes. These defects may be readily observed by referring to figs. 89 and 90.

What has been said, to date, of the renal vascular patterns in normal and ischemic states was found to apply in a roughly equivalent, though less stringent fashion to the vascular patterns in the liver. Thus it was found that x-rays of contrast-injected whole livers, magnified 2 or 3 times, did not reveal more than suggestive changes, if any, in ischemic as compared to normal livers. On the other hand, 700 micron section x-rays of such experimental and control livers showed demonstrable differences. The difficulty lay, however, in the degree of contrast and definition attainable, and it was felt that a great deal more could be shown by alternative methods. Barclay (1951) gives an excellent discussion of the radiological approach to this matter of microscopic detail. He modified the technique developed by Bohatyrtschuk in 1944, who used extremely fine-grain, Lipmann photo-:graphic plates of his own manufacture. In essence, this technique of microarteriography utilized thin tissue sections (ca 200 microns) which were exposed for longish intervals on very slow, fine-grain plates

to the ultra-soft (Grenz) x-rays emitted from a specially constructed x-ray tube with a beryllium window. The technique involved costly apparatus and was fraught with technical difficulties, not the least of which was the need for preventing dessication of the tissue during the long exposure, and the adverse effect of even minor vibrations in the environs. It was decidedly not a procedure applicable to large-scale investigations. (In the future, radioactive isotopes with the necessary emission spectrum for this type of work (e.g. Thulium^{170}oxide) may dispense with the expensive windowed x-ray tube (cf. Mayneord, 1952)). It was decided to investigate the utility of direct photography on cleared thick sections of tissue, excluding the use of x-rays other than for the estimation of grosser changes in the vascular tree.

The formalin-fixed liver and kidneys were sectioned on the freezing microtome at 50, 250 and 700 microns, the liver sections being obtained as horizontal shavings from the broadest and thickest (median) lobe and the kidney sections obtained in longitudinal slices within 800 microns distance from the sagittal (or hilar) plane. The 700 micron sections were stored in formalin for x-ray studies. The 50 and 250 micron sections were dehydrated, cleared and mounted on glass slides in a manner to be discussed under general methods.

A 250 micron section of normal kidney, injected with bismuth contrast medium, is shown in fig. 86. It will be apparent that, even at 10 magnifications, far greater detail is possible by this means than by radiography.

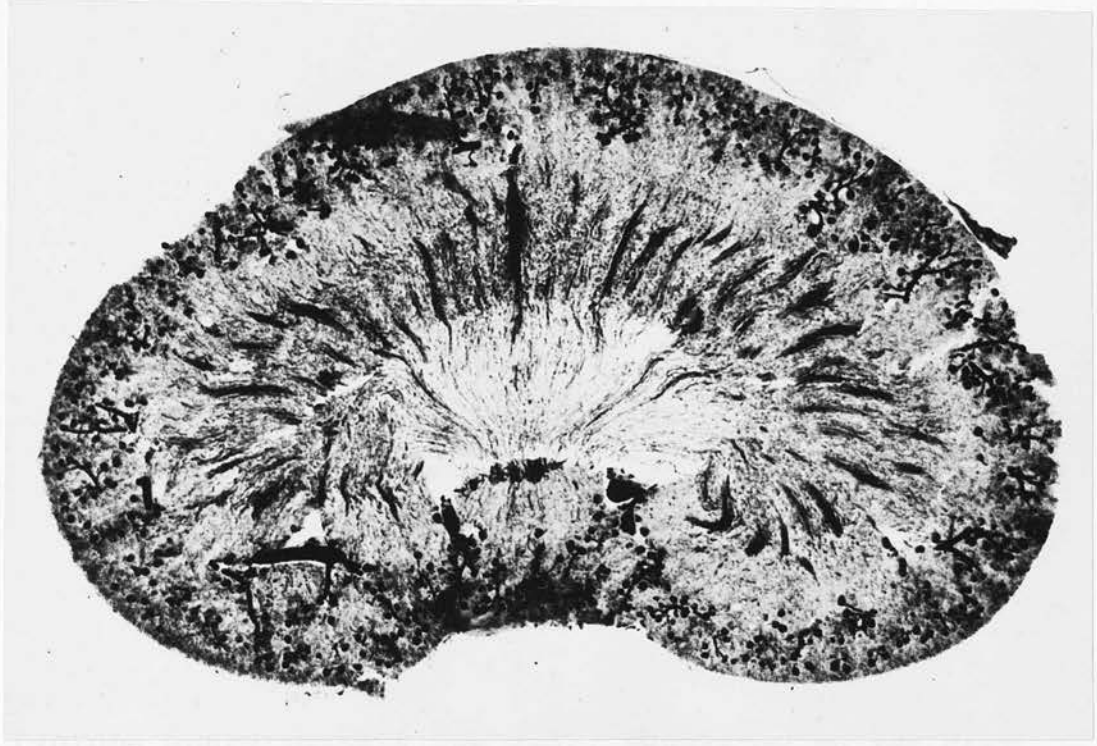


Fig. 86. Normal rat kidney. Photoarteriograph, X10. Bismuth contrast medium, 250 micron section. Note the markedly improved detail obtainable by this technique in comparison to the microradiographs of whole and sectioned kidneys (figs. 87 and 89).

However, fine details of the peritubular capillary net-:work, etc., require even thinner sections and higher magnifications.

General methods.

The methods described below were used routinely

through many phases of the over-all investigations of Section IV and apply in their entirety to the experiments detailed in Section V.

Preparation of the bismuth contrast medium.

150 grams of C.P. bismuth oxychloride were weighed out on a rough balance and dissolved in the smallest possible volume of concentrated hydrochloric acid (about 225 ccs.). The solution was then poured slowly into a large (ca 5 litre) volume of rapidly agitated tap water and the resulting fresh precipitate of bismuth oxychloride was allowed to settle for 1 hour or until it had cleared 4/5ths of the total volume. The supernatant fluid was then aspirated off and water added up to the original level. The wash was repeated once more and the supernate discarded. The sediment and some water were then transferred to a 1 litre graduated cylinder (volume approximately 800 ccs), topped-up to the brim of the cylinder and allowed to settle to approximately 400 ccs. The supernate was aspirated off and replaced once more with tap water. After re-settling to the 400 cc level or less, the final aspiration was performed and the suspension volume was adjusted to a total of 500 ccs. This gave a bismuth oxychloride concentration of 150 grams in 500 ccs, or roughly 30% W/V. The suspension was then shaken well and transferred to a

Townson-Mercer "top-drive macerator" where it was agitated at 10,000 r.p.m. for 5 minutes. At this time, 0.5 ccs of Ilford Wetting Agent were added and the agitation was continued for a further 5 minutes. Then 0.5 ccs of n-octyl alcohol were added, blended momentarily, and 50 grams of dehydrated normal human plasma were incorporated. The mixture was then blended for a further 5 minutes and adjusted with n-octyl alcohol, drop by drop, until excessive foaming was overcome (approximately 0.2 ccs were required at this stage). The mixture was stored in the dark in two 350 cc bottles, with added glass beads. For use it was hand-shaken until the sediment had lifted from the bottom of the bottle, following which it received 10 minutes agitation on a mechanical shaker.

The bismuth contrast medium was shown to have an apparent viscosity of 1.5 at room temperature, compared to 3.0 for pooled, whole human blood. The estimations were performed in an Ostwald Viscometer and the coefficients of viscosity were calculated from the following formula:

$$\frac{V}{t} = \frac{3.14 \times g \times d \times h \times a^4}{8 \times l \times Z}$$

where V = volume (2 ccs)
t = seconds
g = gravity (981)
d = density (blood = 1.06, bismuth = 1.27)
h = mean height (4 cms)
a = radius of the capillary (0.15 cms)
l = length of the capillary (4 cms)
Z = coefficient of viscosity.

This demonstration of a viscosity less than that of blood was instrumental in the choice of an injection pressure roughly equal to that of the systolic arterial pressure in the rat (100 to 130 mm Hg range).

Procedure for injection of the contrast medium.

The animals were prepared by segregating them three days in advance with alcohol and anti-coagulant in the drinking water as described in Section IV. On the day of operation they were given 10 mgs of hexa-:methonium bromide intramuscularly 10 minutes prior to injection. They were then treated as detailed in Section IV, general methods. After opening the rat under deep ether anesthesia from pubis to manubrium sterni, the polythene cannula was tied into place in the descending thoracic aorta. The aorta and its vascular areas were then flushed with heparinized saline for approximately 30 seconds, a clamp was applied just above the bifurcation of the aorta, the

left renal vein was nicked open and the contrast medium was run in at a pressure of 120 mms of mercury. After flow had practically ceased, the kidneys were ligated at the hila and a ligature was slipped over the entire liver and tied down tightly over the porta hepatis and hepatic vein. Kidneys and liver were then dissected free and one kidney and most of the liver were placed immediately into 10% formalin. The other kidney and a lobe of the liver were utilized for histology as previously described (Section IV).

Preparation of the tissues. The ligated liver and kidney were allowed to fix for 24 hours in 10% formalin and were then blotted free of excess surface moisture and x-rayed lying directly on the paper envelope of the x-ray film. The tissues were then frozen and sectioned at 50, 250 and 700 microns, the 700 micron section being x-rayed in the same manner as the whole organ. The 50 and 250 micron frozen sections were floated into the formalin-filled containers which originally contained the tissues. From formalin, each section was floated into a large bowl of methylated spirit for 5 minutes and thence onto a microscope slide, where it was blotted firmly with filter-paper, using a rolling, continuous motion from one end, to affix the section uniformly to the

slide. The section was then dehydrated for 5 minutes in absolute alcohol and finally immersed in xylol until clear (usually 3 to 5 minutes). Cover-slipping with Canada balsam mounting-fluid completed the operation and the slides were set aside for a week or so to allow partial hardening of the mount.

Radiographic and photographic techniques. Radiographic procedures for the livers, kidneys and sections therefrom were as follows: (a) with Ilford Contrast Dental Film, 15 P.

700 micron sections.	100 cms tube to film distance, 50 Kilovolts, 150 milliampere seconds.
Whole kidney.	100 cms distance, 50 K.V., 200 m.a.s.
Whole liver.	100 cms distance, 55 K.V., 250 m.a.s.

(b) with Kodak Crystallex High Contrast Film.

700 micron sections.	100 cms distance, 50 K.V., 50 m.a.s.
Whole kidney.	100 cms distance, 50 K.V., 75 m.a.s.
Whole liver.	100 cms distance, 50 K.V., 100 m.a.s.

After a brief preliminary microscopic examination of the 50 and 250 micron sections they were submitted for photographic enlargement along with the x-ray films of the whole organs and their 700 micron sections. The films of whole livers were enlarged 3 times directly onto printing paper, those of whole kidneys were enlarged 6 times in similar fashion and those of the 700 micron sections of both organs were brought up to 10 magnifications in the same manner. Fifty micron sections of the organs were enlarged 30 times upon printing paper and the 250 micron sections were similarly printed at 10 and 20 magnifications. For purposes of publication, however, the mounted sections have been enlarged first onto photographic plates and subsequently printed in contact fashion, reversing the fields of contrast to give them their natural appearance.

Experiment 1; arteriographic studies in acute carbon tetrachloride intoxication.

Procedure. A total of 67 rats, comprising experimental groups V, W and Y were employed; 35 experimental and 32 control. For no known reason the renal peri-tubular capillaries did not fill adequately with contrast medium in the control animals of the 43 rats

of group Y, and the renal observations were, of necessity, restricted to interpretations of the results from the 24 rats of groups V and W. The intralobular circulation of the liver was found to be adequately outlined in the controls of all three groups and thus the hepatic observations were based on the findings in all animals. The 67 rats were prepared on the reduced food-intake, alcohol-anti-:coagulant regimen for 3 days, then 35 animals were given an anesthetic dose of carbon tetrachloride and animals were sampled from each group at various intervals thereafter. (Note, the thick radiographic sections of groups V and W were cut at 1500 microns. Thick (250 micron) photographic sections were not prepared in these kidney studies).

Results. A. Kidneys. The renal changes are recorded in Table 10.

Table 10.

Incidence & Changes in the Renal Vasculature in CCl_4 Poisoning.

	Time after CCl_4	No. of Rats	Whole organ x-ray.	1500 micron x-ray.	50 micron photo.
Expt.	4 hrs	2	Norm.	? Spasm(2)	Spasm (2)
Cont.	0	2	Norm.	Norm. (2)	Norm. (1)
Expt.	5 hrs	4	Norm.	? Spasm(4)	Spasm (4)
Cont.	0	3	Norm.	Norm. (3)	Norm. (3)
Expt.	9 hrs	2	Norm.	? Spasm(2)	Spasm (1)
Cont.	0	2	Norm.	Norm. (2)	Norm. (2)

Table 10 contd.

	Time after CCl_4	No. of Rats	Whole organ x-ray.	1500 micron x-ray.	50 micron photo.
Expt.	24 hrs	2	Norm.	Norm. (2)	Spasm (2)
Cont.	0	2	Norm.	Norm. (2)	Norm. (2)
Expt.	30 hrs	3	Norm.	Norm. (3)	Norm. (3)
Cont.	0	2	Norm.	Norm. (2)	Norm. (2)

From these recorded observations it is apparent that no changes are detectable in x-rays of the whole kidneys throughout the intervals studied (see figs. 87 and 88). The same observations were made in earlier studies with pituitrin. In the x-rays of the 1500 micron sections of the CCl_4 kidneys, there is a suggestion of a barely perceptible obliteration of many afferent arterioles, on the strength of which, the findings have been entered as "? Spasm" in Table 10. This questionable change is illustrated by comparison of figs 89 and 90, control and 4 hour specimens respectively. These changes are apparently present uniformly between the 4 and 9 hour intervals but cannot be seen at 24 hours. In order to ensure an unbiased interpretation of the radiographs, they were sorted by an independent observer into two separate groups, those showing numerous fine terminal branches and those with a marked reduction in this feature. The result of this division was identical to the results recorded in Table 10.

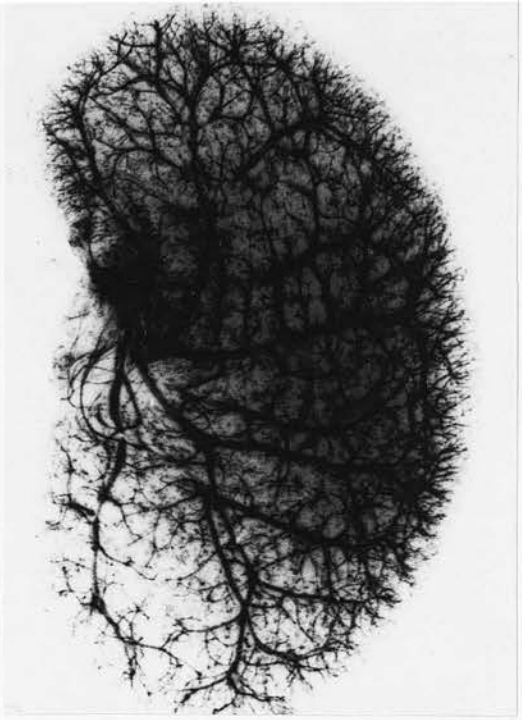


Fig.87. Control whole kid-:ney.Radioarteriograph X6, using bismuth contrast medium.For comparison with fig. 88.

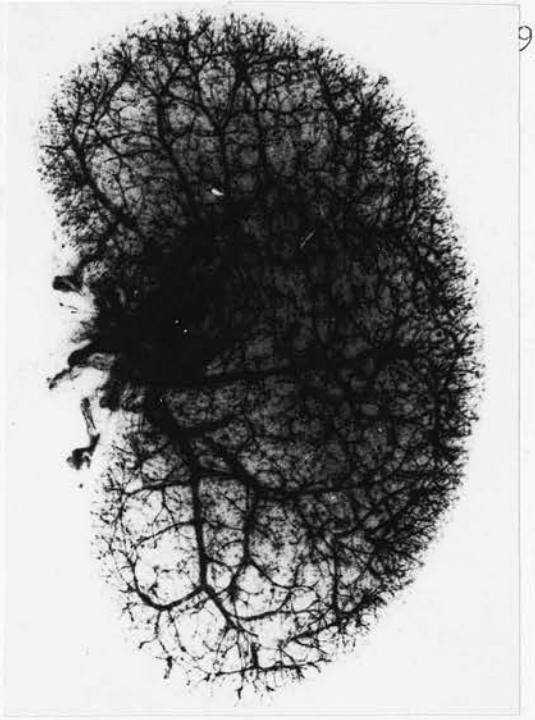


Fig.88. 4 hrs CCl_4, whole kid-:ney. Radioarteriograph X6, with bismuth contrast medium. There are no obvious changes when compared with fig. 87.

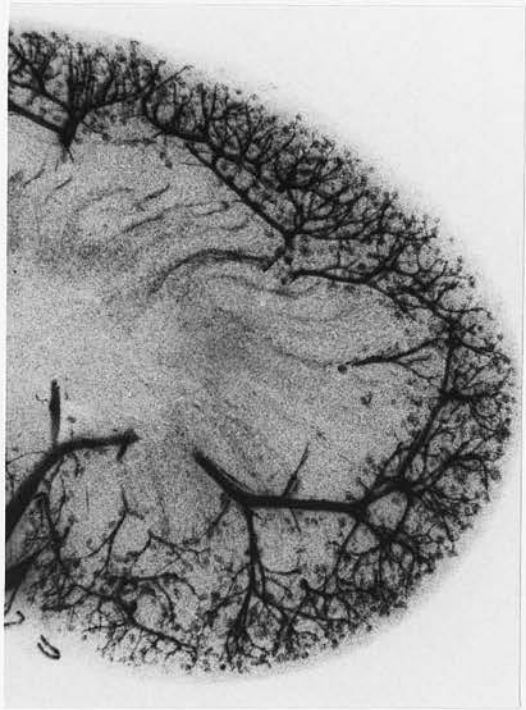


Fig.89.Control 1500 micron kidney section.Radioarterio-:graph X10.Note the suggest-:ion of very fine(afferent arteriole) branches from the interlobular arteries.
Bismuth contrast medium.

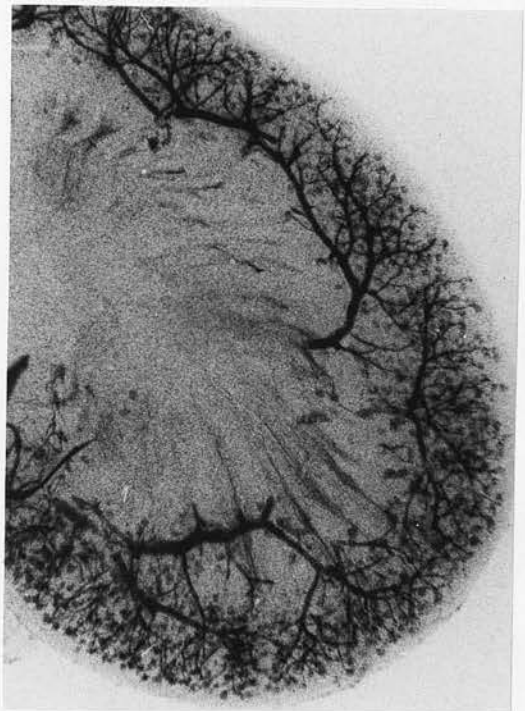


Fig.90. 4 hrs CCl_4, 1500 micron kidney section. Radioarterio-:graph X10. A decrease in the total number of fine terminal branches is suggested.(See fig.89)

The findings elicited from the 30 X photo-arteriographs of the best-injected areas of 50 micron unstained sections are far more impressive. These changes consist of a marked, patchy reduction in filling of the peri-tubular capillaries between 4 and 24 hours, plus a definite increase in the diameters of the glomeruli in the ischemic as opposed to the normal kidneys.

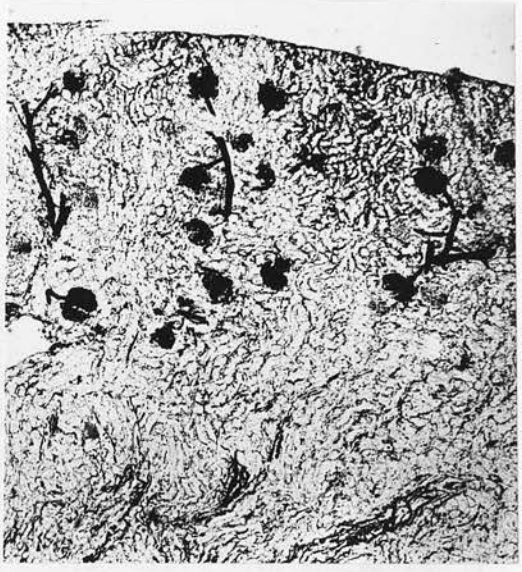


Fig.91. Photoarteriograph, X30. Normal control. 50 micron, unstained section of kidney showing glomeruli and peritubular capillaries well filled with the bismuth contrast medium.

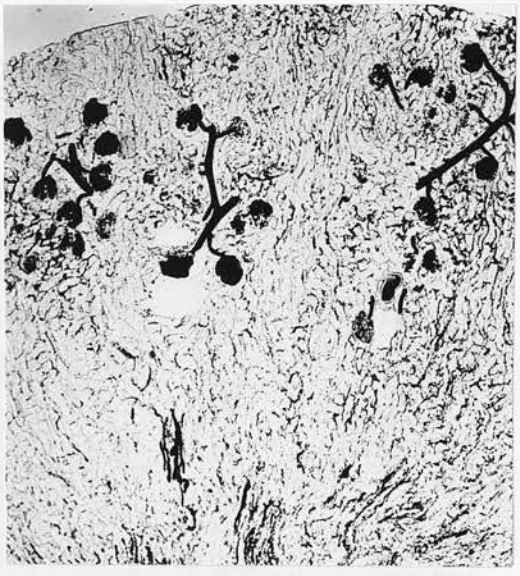


Fig.92. Photoarteriograph X30. 4 hrs after CCl_4, 30 micron, unstained section of bismuth-injected kidney. Note the patchy absence of contrast medium in the capillaries.

By caliper measurement of all glomeruli in the photo-arteriographs between 4 and 24 hours, the average ratio of diameters of the CCl_4 glomeruli to those of control glomeruli is 1.2/1. The kidney sections appear

normal 30 hours after CCl_4 intoxication. There is failure of adequate contrast filling in 1 out of 11 control kidneys and overfilling in 1 out of 10 experimental kidneys between 4 and 24 hours, giving a maximum reliability in each instance of the order of 90%. The changes are well illustrated in figs. 91, 92, 93, 94 and 95; control, 4, 9, 25 and 30 hour specimens respectively.

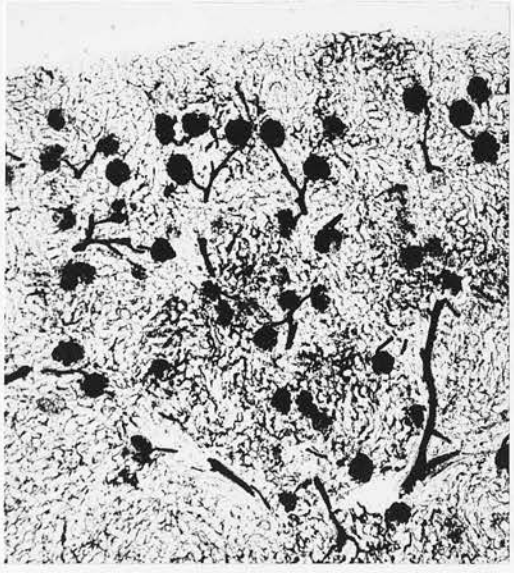


Fig.93. Photoarteriograph X30. 9 hrs after CCl_4. 50 micron section of kidney showing changes similar to those seen at 4 hours.

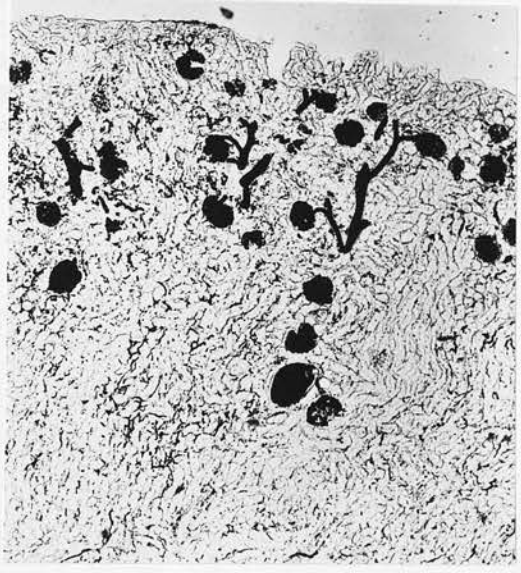


Fig.94. Photoarteriograph X30. 24 hrs after CCl_4. 50 micron kidney section. The changes resemble those seen at 4 and 9 hours (figs. 92 and 93).

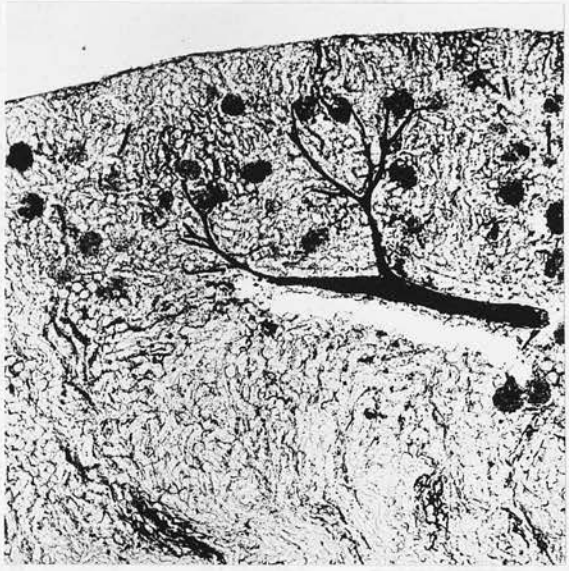


Fig.95. Photoarteriograph X30. 30 hrs after CCl_4. 50 micron unstained kidney section. The patchy failure of injection in the cortical peritubular capillaries is no longer present.

Glomerular counts performed on these 50 micron sections at 150 magnifications show no significant differences between experimental and control kidneys in the relative numbers of glomeruli injected with contrast medium to those uninjected.

B.Livers. In the 35 carbon tetrachloride liver specimens there is a uniform reduction in the intra-:lobular (or sinusoidal) inflow of bismuth contrast medium from 1 hour to 36 hours as seen in the 50 and 250 micron sections. Whole liver x-rays show no obvious changes throughout these intervals, but the changes can be visualized readily in all 1500 and 700 micron sectional x-rays. The findings are detailed in Table 11.

Table 11.

Incidence & Alterations in Hepatic Vasculature after CCl_4

	Time after CCl_4	No. of Rats.	Whole organ x-ray.	Thick sect. x-ray.	50 & 250 micron photo-graphs.	Incid-ence.
Expt.	1 hr	4	Normal	Ischemia	Ischemia	4
Cont.	0	3	Normal	Normal	Normal	3
Expt.	4 hrs	6	Normal	Ischemia	Ischemia	6
Cont.	0	6	Normal	Normal	Normal	6
Expt.	5 hrs	4	Normal	Ischemia	Ischemia	4
Cont.	0	3	Normal	Normal	Normal	3
Expt.	6 hrs	4	Normal	Ischemia	Ischemia	4
Cont.	0	4	Normal	Normal	Normal	4
Expt.	8 hrs	2	Normal	Ischemia	Ischemia	2
Cont.	0	2	Normal	Normal	Normal	2
Expt.	10 hrs	4	Normal	Ischemia	Ischemia	4
Cont.	0	4	Normal	Normal	Normal	4
Expt.	12 hrs	2	Normal	Ischemia	Ischemia	2
Cont.	0	2	Normal	Normal	Normal	2
Expt.	24 hrs	4	Normal	Ischemia	Ischemia	4
Cont.	0	4	Normal	Normal	Normal	4
Expt.	30 hrs	3	Normal	Ischemia	Ischemia	3
Cont.	0	2	Normal	Normal	Normal	2
Expt.	36 hrs	2	Normal	Ischemia	Ischemia	2
Cont.	0	2	Normal	Normal	Normal	2

Radioarteriographs of the whole livers in a control and a 4 hour experimental animal are shown in figs. 96 and 97 respectively.

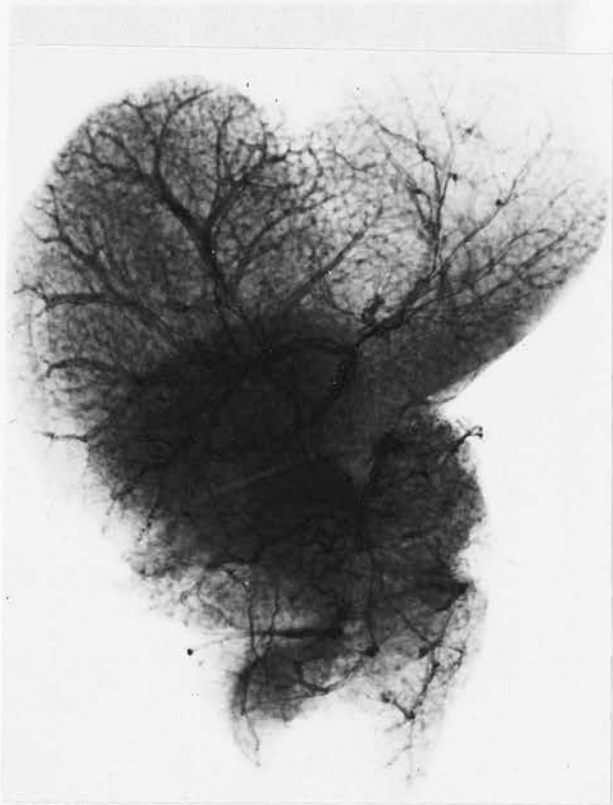


Fig.96. Radioarteriograph X3. Control whole liver with bismuth contrast. See fig. 97.

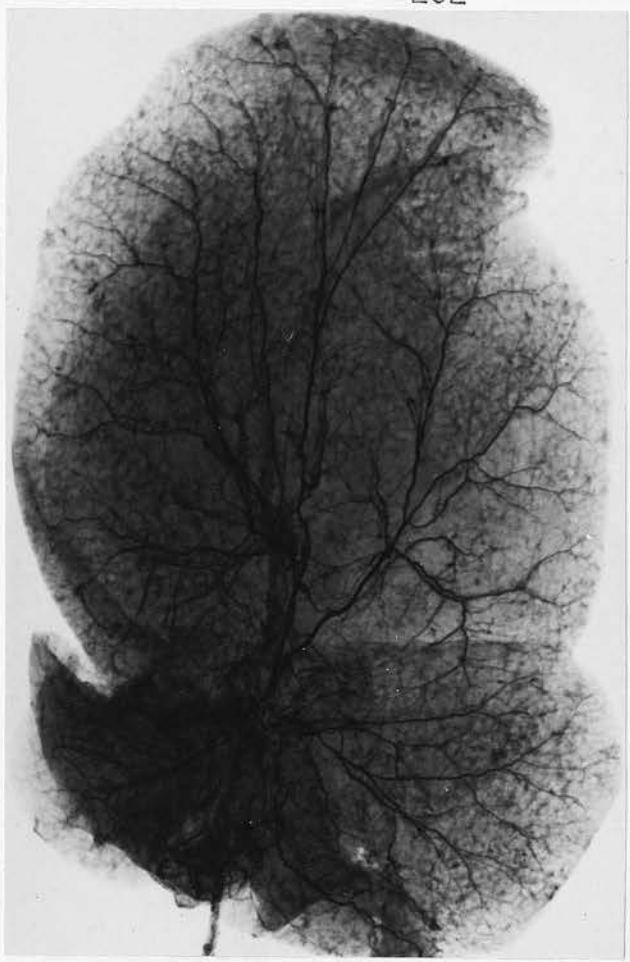


Fig.97. Radioarteriograph X3. Whole liver, 4 hrs after CCl_4. No obvious changes can be seen in comparison with fig. 96.

The ischemic state of the livers of animals exposed to carbon tetrachloride is shown most clearly by means of photoarteriography, the field for photography being taken from the area most fully injected with contrast medium. Figs. 98 to 102 represent, successively, control, 1, 4, 6 and 36 hour CCl_4 specimens from 250 micron unstained sections at 20 magnifications. There is noted a sharp decrease

Fig.98. Rat liver, 250 microns. Photoarteriograph X20. Control. Bismuth contrast medium. The fine black lines in the portal tracts represent branches of the hepatic artery; coarse vessels are portal vein radicles. Note the uniform sinusoidal pattern and the thin portal tracts.

Fig.99. Rat liver, 250 microns. Photoarteriograph X20. 1 hour after CCl_4. No change is seen in the major vessels. Note the marked blanching of the intra-lobular circulation and the prominent arteriolar leashes in the portal areas, as compared with fig. 98.

in contrast injection of the intralobular circulation from the 1st hour onwards. The arteriolar leashes in the portal tracts are distended and well displayed as a consequence of the impeded sinusoidal flow. Absolute ischemia is never seen.

Fig.100. Rat liver, 250 microns. Photoarteriograph X20. 4 hour specimen. The picture is identical to the 1 hour change as seen in fig. 99.

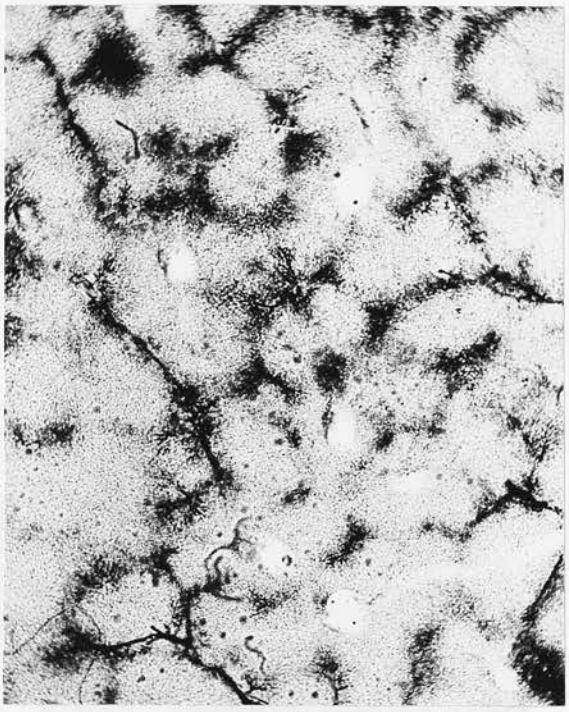


Fig. 101.Rat liver, 250 microns. Photoarteriograph X20. 6 hour specimen. Sinusoidal ischemia continues and the picture is as seen at 1 and 4 hrs (figs. 99 and 100).

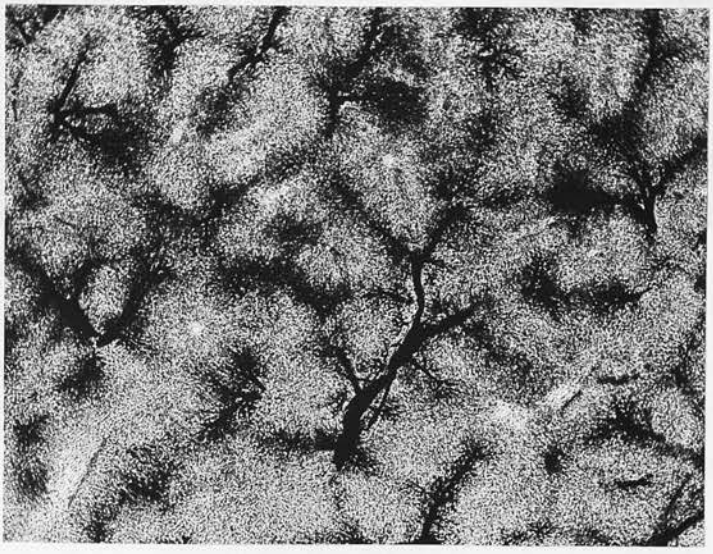


Fig.102. Rat liver, 250 microns, bismuth contrast. Photoarteriograph X20. 36 hrs after CCl_4 . Note that bismuth particles are found throughout the lobule to an increased extent, but there is still undue prominence of the portal arteriolar leashes and the picture is not completely normal.(Compare with fig.107).

Similar findings are seen in 30-times enlargements of the unstained 50 micron sections taken at the same time intervals, as illustrated in figs. 103 to 107.

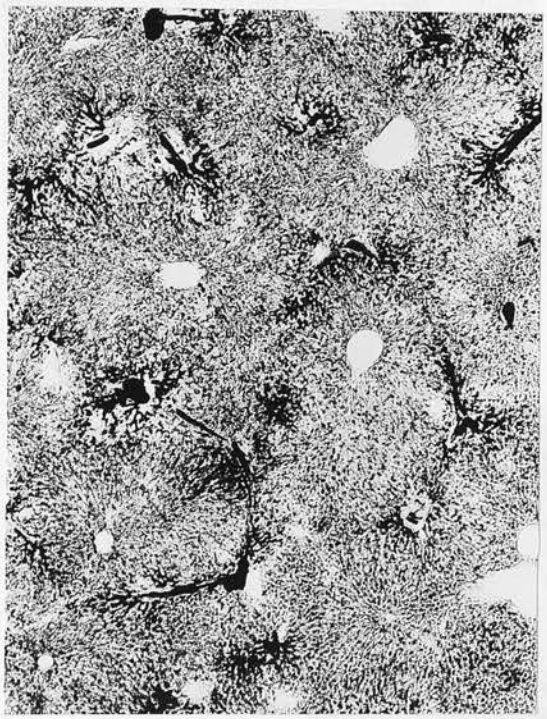


Fig. 103.Rat liver, 50 micron section X30. Photoarterio-:graph. Control specimen. The portal and intralobular circulations are well outlined by bismuth particles.

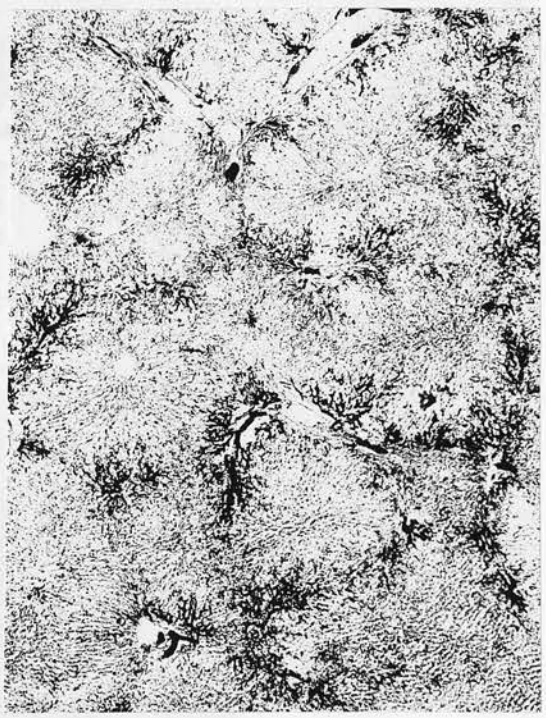


Fig.104. Rat liver, 50 micron section X30. Photoarterio-:graph. 1 hour after CCl_4. Note the lessened degree of intralobular injection and prominence of arteriolar leashes in portal areas.

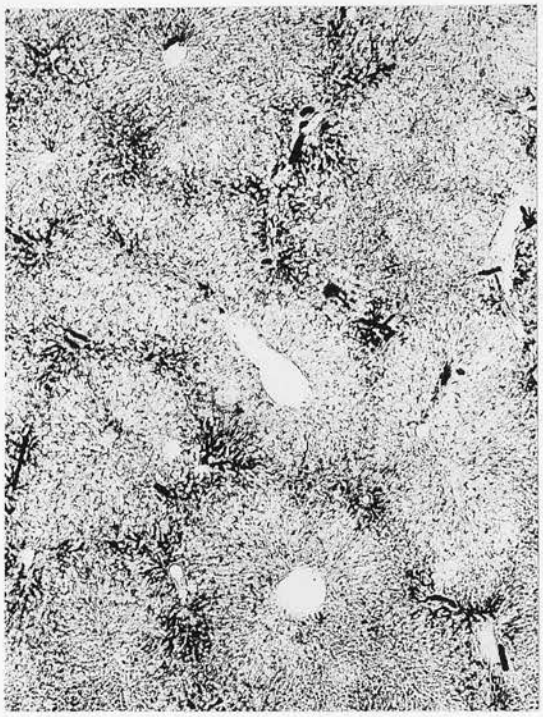


Fig.105. Rat liver, 50 micron section X30. Photoarterio-graph. 4 hrs after CCl_4. The features are identical to those of fig. 104 at 1 hour.

Fig.106. Rat liver, 50 micron section X30. Photoarterio-graph. 6 hrs after CCl_4. Very little bismuth now enters the sinusoids. The arteriolar leashes in the portal tracts are grossly distended.

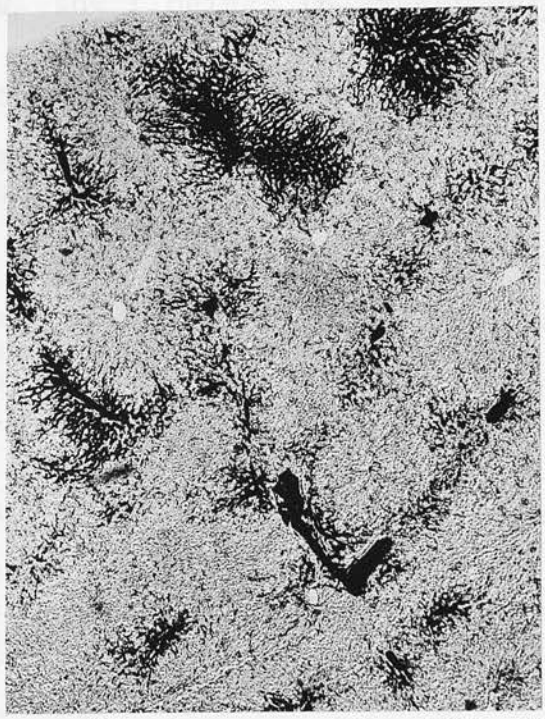


Fig.107. Rat liver, 50 micron section X30. Photoarterio-graph with bismuth contrast. 36 hrs after CCl_4. There is more contrast medium in the lobular sinusoids than seen at 6 hours (fig.106), but it is of patchy distribution. The arteriolar leashes are very well defined in the portal areas. (Compare with fig. 102).

These illustrations reveal in most striking fashion the impedence in the intralobular circulation resulting from carbon tetrachloride poisoning. In addition, as a result of partial sinusoidal obliter-:ation, the anatomy of the pre-sinusoidal vascular tree is well defined. The hepatic artery and portal vein radicles can be seen to break out in a feathery leash of arterioles to supply the several surrounding lobules. This feature is even more readily appreciated from the 250 micron sections when viewed under a stereoscopic dissecting microscope.

Summary of the findings in Experiment 1.

1. The kidneys react to acute carbon tetrachloride intoxication by focal, patchy ischemia of the peritubular capillaries, most striking in the cortical zone. There is no demonstrable change in the lumina of larger renal arteries.

2. Such changes are best visualized by direct photo-:arteriography with the tissues magnified by at least 30 times. There is no apparent change in the whole-kidney x-ray films.

3. The glomerular tufts are slightly larger in the ischemic carbon tetrachloride kidneys than in the control kidneys, whereas there is no decrease in the ratios of injected/uninjected glomeruli in the experimental as opposed to the control group.

4. Sectional kidney x-rays (1500 microns) show a suggestion of focal obliterative changes at the pre-arteriolar level, but this cannot be ascertained with certainty. As noted above, there is certainly no deficiency in the number of glomeruli filled with contrast medium between experimental and controls.

5. Attempts to investigate the renal vascular tree at 1 hour failed due to agglomeration of the bismuth particles. Contrast medium is found to be excluded from the peritubular capillaries of both experimental and control kidneys. This agglomerative tendency on the part of the bismuth oxychloride is difficult to understand, since it varies from one preparation to the next. The medium in question was freshly prepared two days prior to use, yet it would not pass the efferent arterioles. It is possible that variations in human plasma proteins (e.g. lipid and protein contents) may alter the stability of the suspension. Media employed in the earlier experimental groups ran readily into the capillaries, viz: fig. 91.

6. Focal renal ischemia at the level of the peri-:tubular capillaries is found from 4 to 24 hours after exposure to carbon tetrachloride and disappears by 30 hours.

7. Due to inadequacy of the bismuth injection medium, the renal arteriographic studies of acute CCl_4 intoxication will require repetition in the future.

8. Radioarteriographs of whole livers show no appreciable differences between the carbon tetrachloride and control groups. There is no obvious change in the lumina of the hepatic artery or the portal vein.

9. Radioarteriographs of 1500 micron sections of liver tissue do show ischemia of the liver lobules, but contrast and definition are poor.

10. Photoarteriographs of 50 and 250 micron sections, enlarged 30 and 20 times respectively, give sharp definition and excellent detail of the finest ramifi-:cations of the hepatic vascular bed. From these studies there is found a severe degree of sinusoidal ischemia between 1 and 36 hours following exposure to CCl_4 as compared to control livers.

11. Such studies also provide an excellent demonstration of the portal tract arteriolar leashes from which the sinusoidal flow is derived. These vessels are grossly dilated in the experimental animals due to the impairment of forward flow in the intralobular circulation caused by the swelling of the liver cord cells lining the sinusoids. (And see Section IV, Experiment 1).

Experiment 2; arteriographic studies in acute mercury poisoning.

Procedure. Seventeen rats of experimental group Z were segregated and given alcohol and anticoagulant in the drinking water plus the standard diet ad lib for 3 days. Nine rats were then injected with 0.5 mgs of mercuric chloride intra-muscularly into the thigh and animals were subsequently sampled from both experimental and control groups at 1, 4, 6 and 9 hours, totalling 4 rats for the first three periods and 5 for the last. Due to a fault in the contrast medium employed, the entire experiment was repeated one week later with a fresh preparation of bismuth oxychloride suspension. In addition, four control and two 4-hour mercury-treated rats were later injected with 20% vermillion medium.

Results. A. Kidneys. The kidneys of the first attempt in group Z failed to fill beyond the efferent arterioles in control and experimental animals alike,

and the results are therefore limited to the second group of 17 rats in the repeat portion of this experiment. No changes are found in the 700 micron and whole-kidney microradiographs during the first 6 hours. At the 9 hour period there is a suggestion of pallor throughout the cortical zone in both the full-thickness and the 700 micron section x-ray films in the mercurial kidneys as compared to the controls. These changes are illustrated in figs. 108 to 111 inclusive.

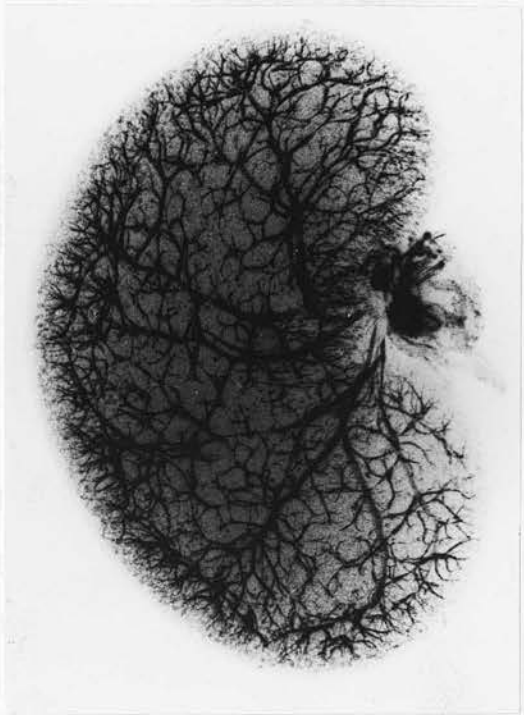


Fig.108. Whole rat kidney. Radioarteriograph, X6. Control specimen, with bismuth contrast medium.

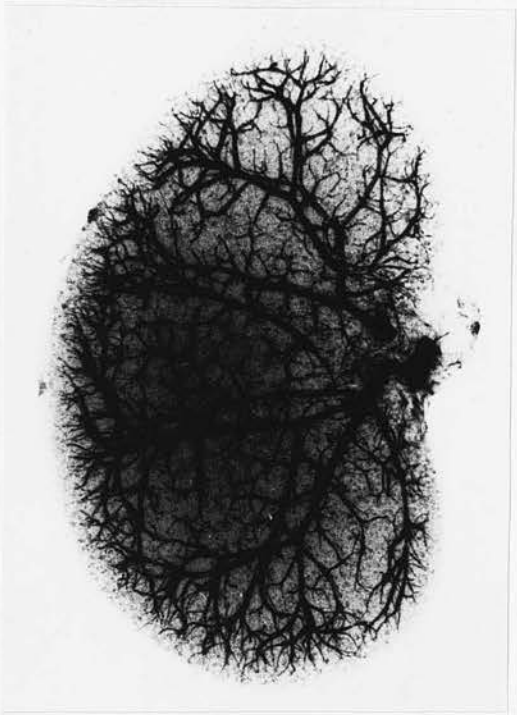


Fig.109. Whole rat kidney. Radioarteriograph, X6. 9 hours after mercury. Note a suggestion of cortical pallor as compared to control.

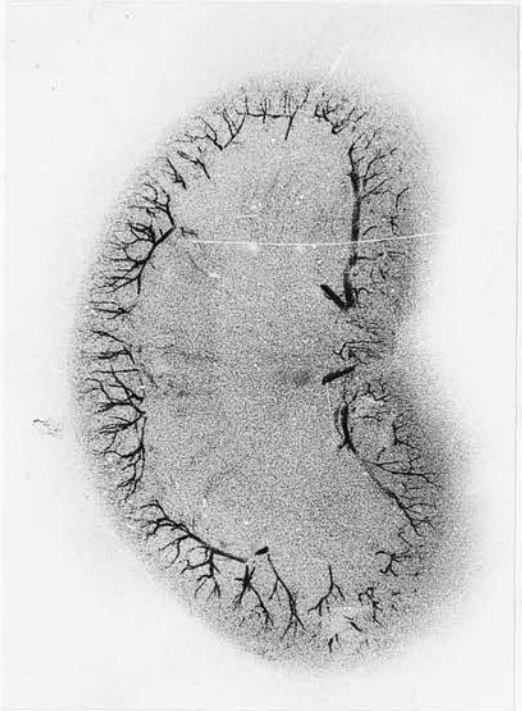


Fig.110. 700 micron kidney section. Radioarteriograph X6. Control as in fig. 108. For comparison with fig. 111.

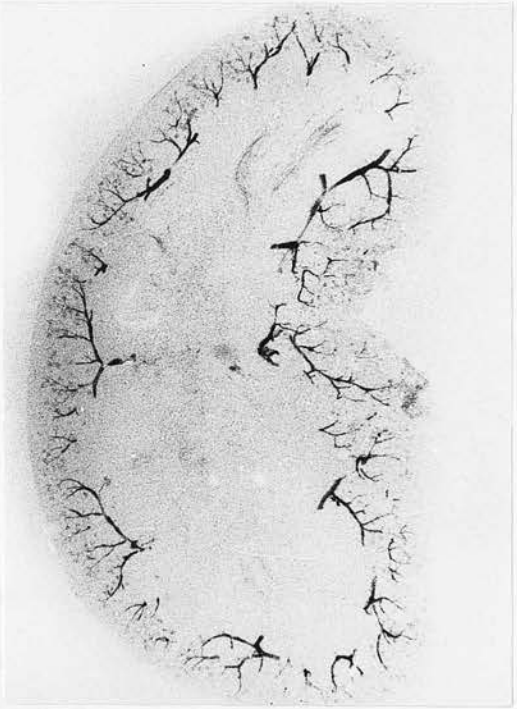


Fig.111. 700 micron kidney section. Radioarteriograph X6. 9 hours after mercury, same kidney as fig. 109. Note the marked decrease in filling of terminal vessels. (c.f.fig. 110).

The new bismuth medium is found to have a finer particle size than its predecessor, with moderately good filling of the peritubular capillaries as seen in 250 micron sections, but there is still a disturbingly high incidence of inadequate contrast-filling in control animals. Two of the 8 control 250 micron sections could pass for mercurial kidneys, giving the results a maximum reliability of 75%. Likewise, one of the mercurialized kidney sections at the 6 hour interval is as well injected as any control. The findings

at the 250 micron level may be summed-up as follows: The peritubular capillaries are well injected with contrast medium at 1 hour. By 4 hours there is widespread ischemia of the capillary network. After 6 hours, one kidney reveals capillary ischemia, the other is well filled. (Compare figs. 112 and 115). 9 hours after mercury poisoning, all 3 kidney sections show extensive, patchy obliteration, both of the peritubular capillaries and of the glomeruli, afferent arterioles and interlobular arteries. Fig. 112 illustrates the control kidney section and figs. 113 to 116 the mercurialized kidney tissue from 250 micron, unstained sections, at 1, 4, 6, and 9 hours respectively in 10-times magnified photoarteriographs.

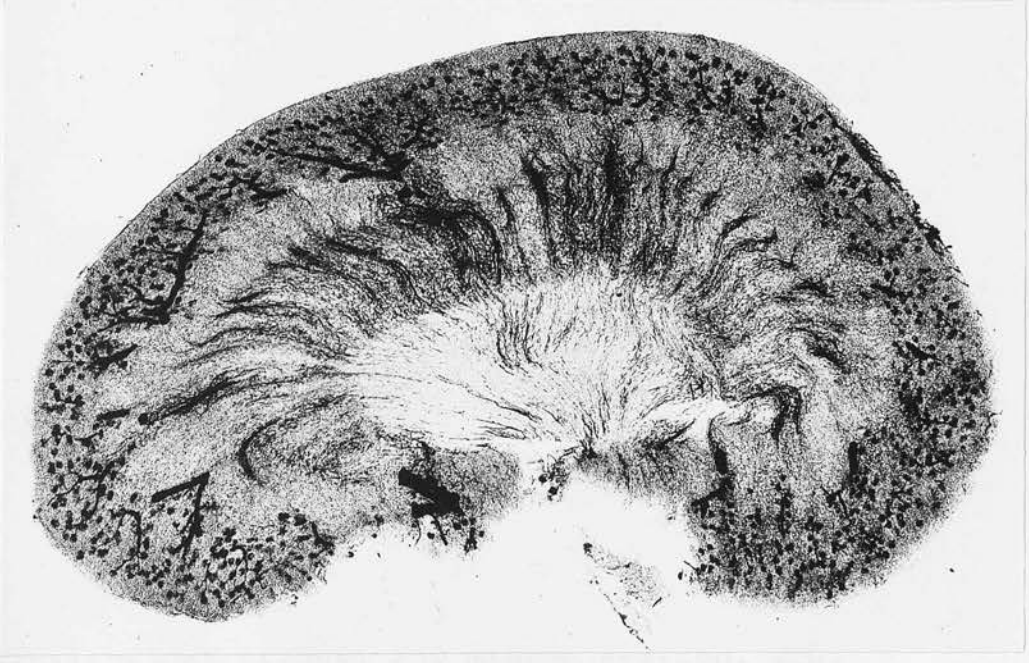


Fig. 112. Rat kidney, 250 micron section, X10. Photoarteriograph of entire section. Control, showing reasonably good filling of the peritubular capillaries with bismuth contrast medium.

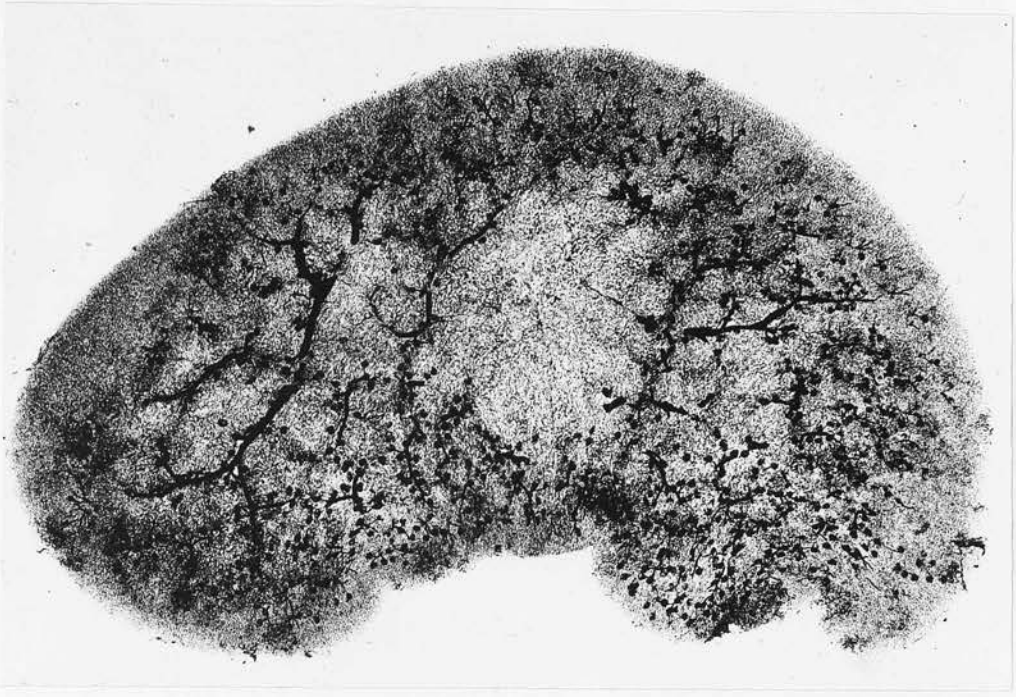


Fig. 113. Rat kidney, 250 micron section, X10. 1 hour after mercury poisoning. Photoarteriograph. Note reasonably good filling of the glomeruli and capillaries. The specimen is quite comparable to the control shown in fig. 112.

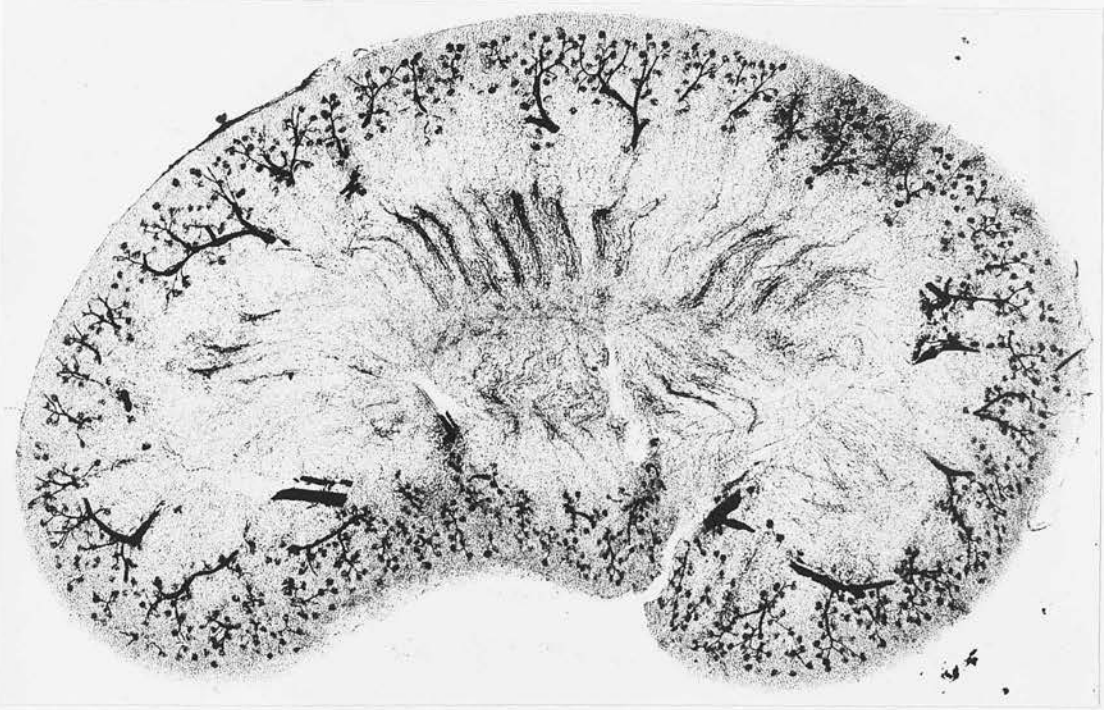


Fig.114. Rat kidney, 250 micron section, x10. Photoarteriograph, 4 hours after mercury poisoning. The peritubular capillaries are markedly ischemic, while the glomeruli appear adequately filled.

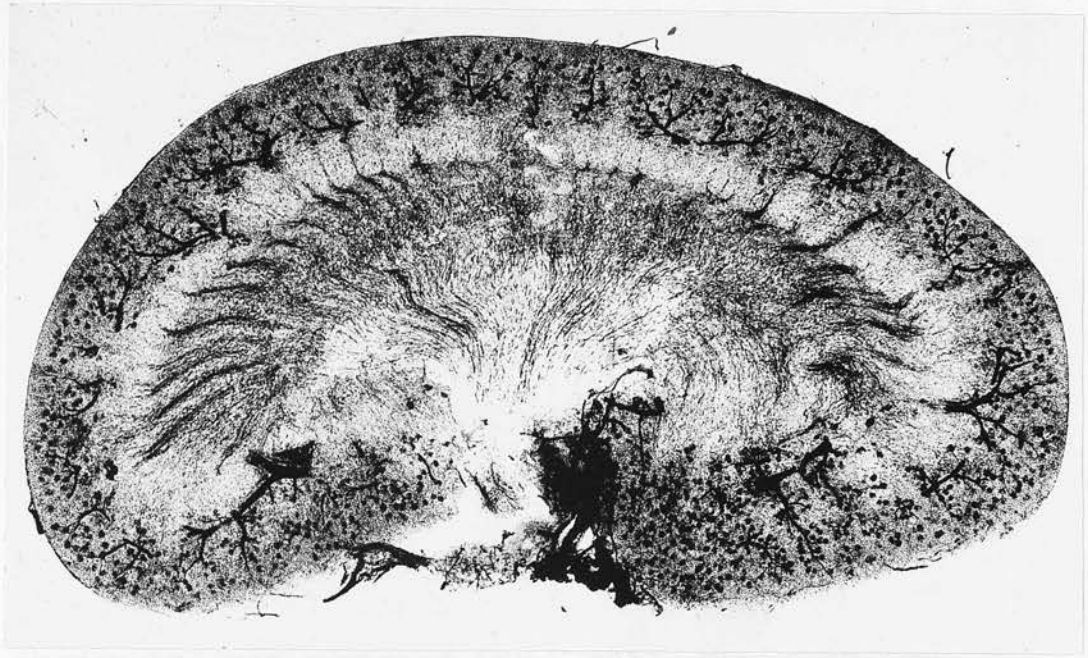


Fig. 115. Rat kidney, 250 micron section, x10. Photoarteriograph, 6 hours after mercury poisoning. Glomerular and capillary filling appear normal as compared to the control (fig. 112).

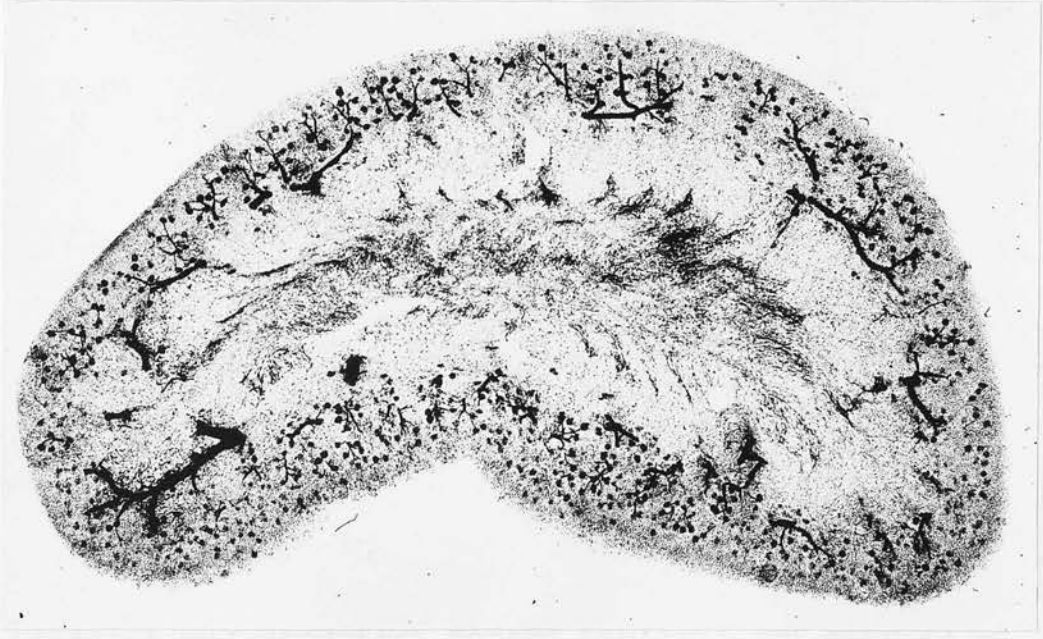


Fig. 116. Rat kidney, 250 micron section, X10. Photoarteriograph, 9 hours after mercury poisoning. Defective filling is observed in many interlobular arteries, afferent arterioles and glomerular tufts. The peritubular capillaries show widespread ischemia.

The bismuth-injected 50 micron sections show insufficient contrast-medium penetration of the peri-:tubular capillaries to permit adequate comparison. On the other hand, using 20% vermillion as contrast medium, there is noted good contrast penetration of the capillary network in all 4 control kidneys and a reduction of filling of this network in the kidneys of the 2 animals given mercuric chloride 4 hours previously. These changes appear to be identical to those seen 4 hours after CCl_4 intoxication when a fine bismuth suspension was employed (fig. 92). They are well illustrated in figs. 117 and 118, representing the control and the 4-hour mercury specimens respectively.

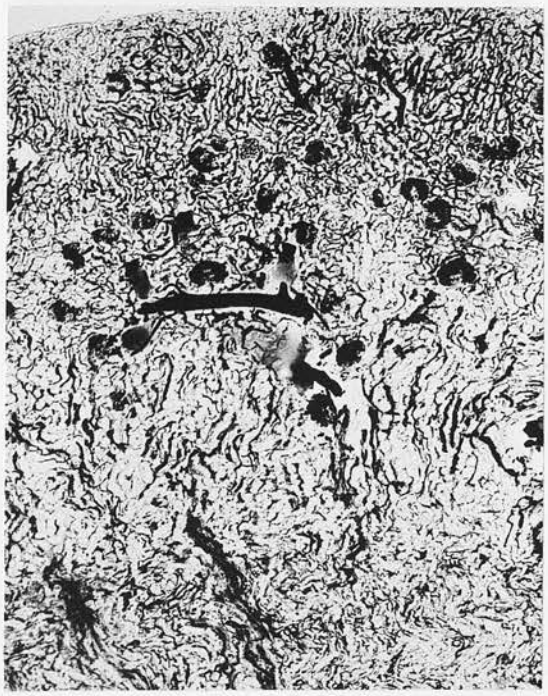


Fig.117. Rat kidney, 50 micron section. Vermillion contrast medium. Photoarteriograph, X30. Control specimen. Note the excellent filling of arterial and capillary structures. For comparison with fig.118.

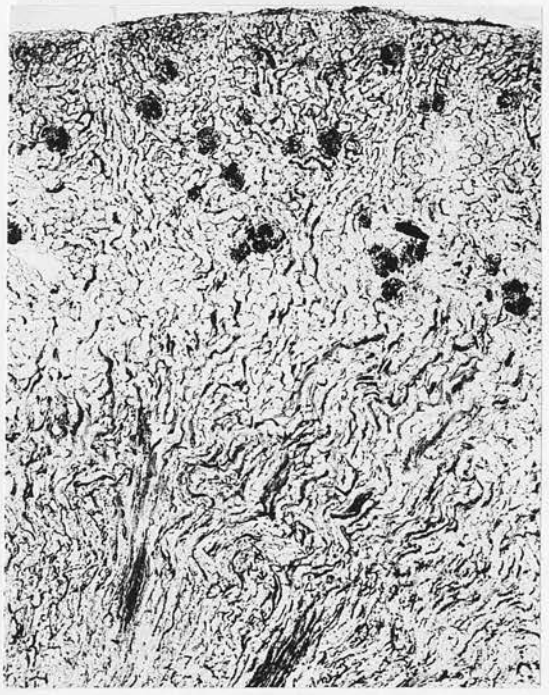


Fig.118. Rat kidney, 50 micron section. Vermillion contrast medium. Photoarteriograph, X30. 4 hrs after mercuric chloride. Note the relative degree of peritubular capillary ischemia as compared with control(fig.117)

B. Livers. The observations on the livers are drawn from the results of both the original and the repeat experiments, since adequate filling is seen throughout all controls. No changes of note can be demonstrated in radioarteriographs of whole livers enlarged twice, as illustrated in figs. 119 and 120, control and experimental respectively.

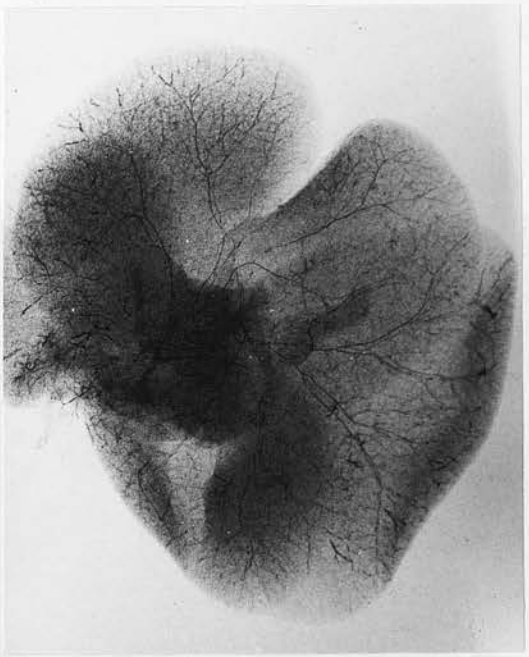


Fig. 119. Rat whole liver. Radioarteriograph, X2. Control specimen. Bismuth contrast medium. Compare with fig. 120.

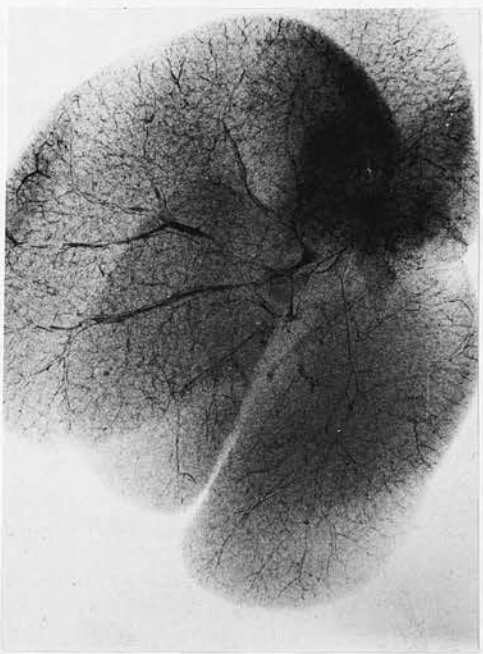


Fig. 120. Rat whole liver. Radioarteriograph, X2. 4 hrs after mercuric chloride. No apparent alteration is seen from fig. 119.

A suggestion of an alteration is apparent in all radioarteriographs of 700 micron sections of the mercurialized livers as opposed to the controls between

1 and 9 hours. This change consists of ischemia of the intralobular circulation, which, as shown in Section IV, Experiment 2, results from swelling of the parenchymal cells and partial obliteration of the sinu-:soids. On the other hand, definition and contrast is far superior in the photoarteriographs of 250 and 50 micron sections of the liver, as was the case in the livers poisoned with carbon tetrachloride. Figures 121 to 126 illustrate the changes observed in 250 micron sections between the control liver and the mercurialized livers at 1, 4, 6, 9 and 9 hours respectively.

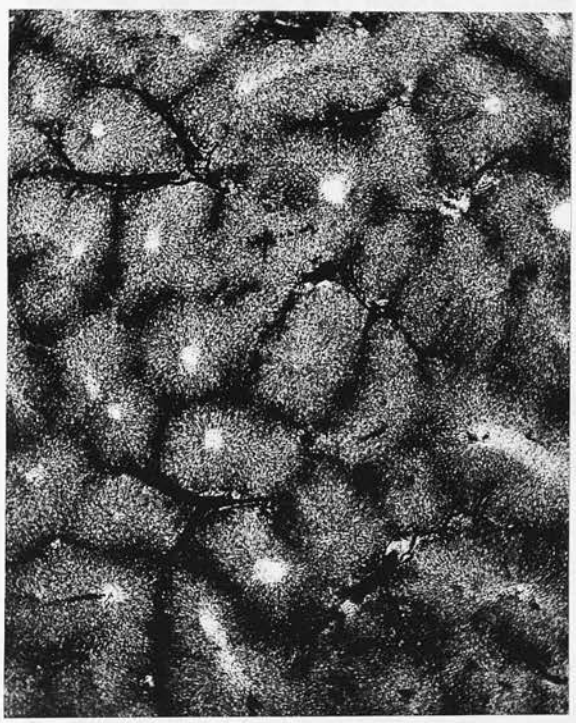


Fig.121. Rat liver, 250 micron section. Photoarteriograph, X20. Control specimen, bismuth contrast medium.

Fig.122. Rat liver, 250 micron section. Photoarteriograph, X20. 1 hr after $HgCl_2$, bismuth contrast. Note marked pallor of the sinusoids due to exclusion of bismuth particles. The art-:eriolar leashes in the portal areas are prominent as a result of impeded flow.

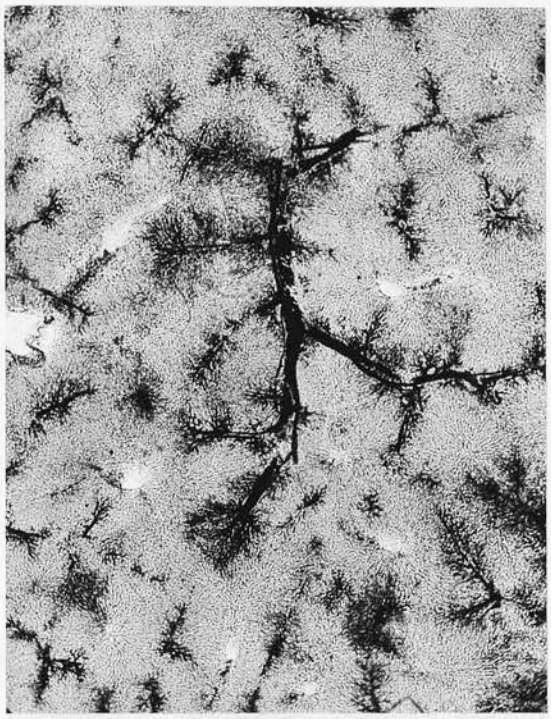


Fig. 123. Rat liver, 250 micron section. Photoarteriograph, X20. 4 hrs after $HgCl_2$. The intralobular ischemia is more pronounced than at 1 hour.

Fig.124. Rat liver, 250 micron section. Photoarteriograph,X20. 6 hrs after mercury. Sinusoidal ischemia and portal hyperemia is present as before, though some bismuth now enters the intralobular vessels.

The 9 hour liver shown in fig. 126 suggests a partial return to a more normal sinusoidal pattern, as compared to another 9 hour experimental liver illustrated in fig. 125, where the ischemia is still extreme. (Three of the remaining livers at 9 hours after mercury are similar to that shown in fig. 125, the fourth being a reasonable facsimile of that illustrated in fig.126). The obstructive pattern seen in vessels in the portal areas is identical to that of the carbon tetrachloride liver.

Fig.125. Rat liver, 250 micron section. Photoarteriograph, X20. 9 hrs after mercury. The picture is very similar to the 6 hour change shown in fig. 124.

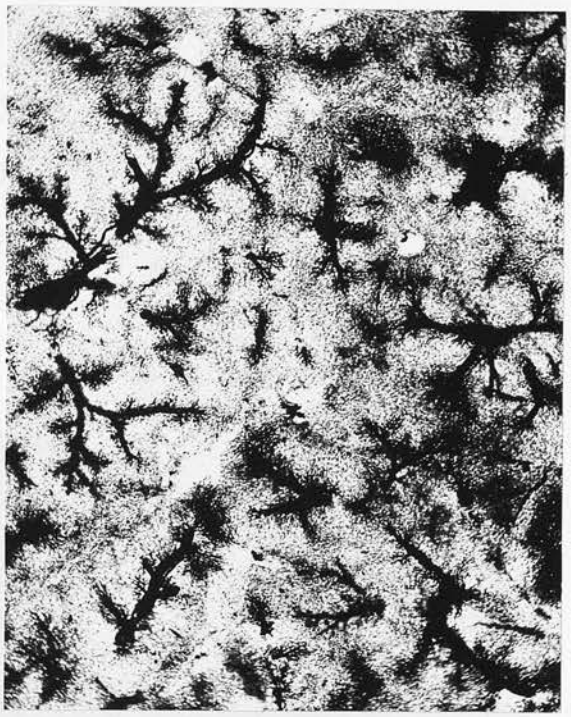


Fig.126. Rat liver, 250 micron section. Photoarteriograph, X20. 9 hrs after mercury. Lobular ischemia is still present, though not so markedly as in the other 9 hr liver (fig. 125).

All the changes illustrated above are apparent in even greater detail in the 50 micron sections magnified 30 times. It should be noted that in all instances where area enlargements are employed, the photographs have been taken through that portion of tissue most heavily infiltrated with contrast medium.

Figures 127 to 131 inclusive illustrate control, 1, 4, 6 and 9 hour mercurial livers respectively.

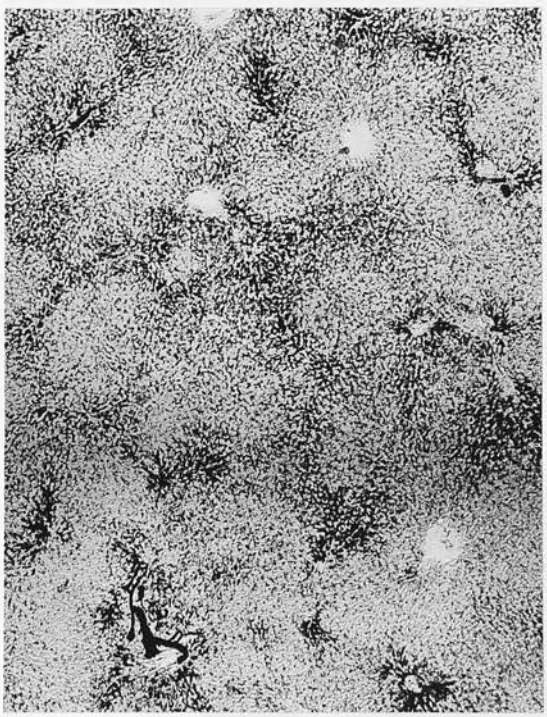


Fig.127. Rat liver, 50 micron section. Photoarteriograph, X30. Control specimen, with bismuth contrast medium outlining the entire intra-lobular circulation.

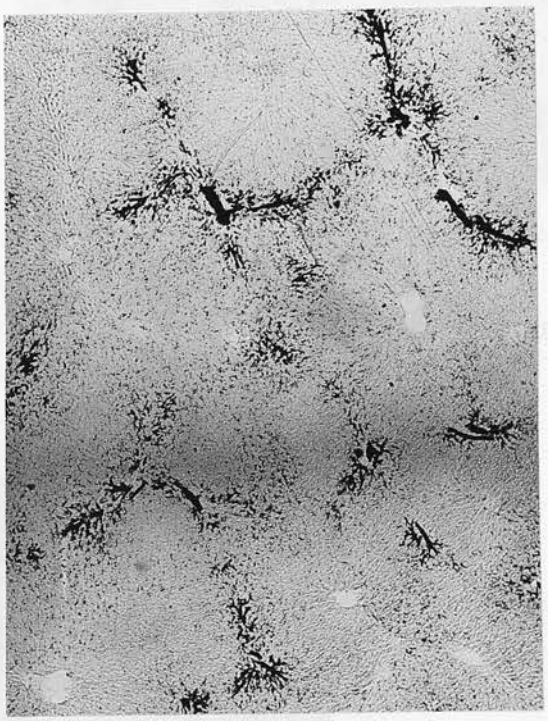


Fig. 128. Rat liver, 50 micron section. Photoarteriograph, X30. 1 hr after $HgCl_2$. Virtually total ischemia of the sinusoids is present. Compare with the corresponding CCl_4 liver at 1 hour (fig. 104).

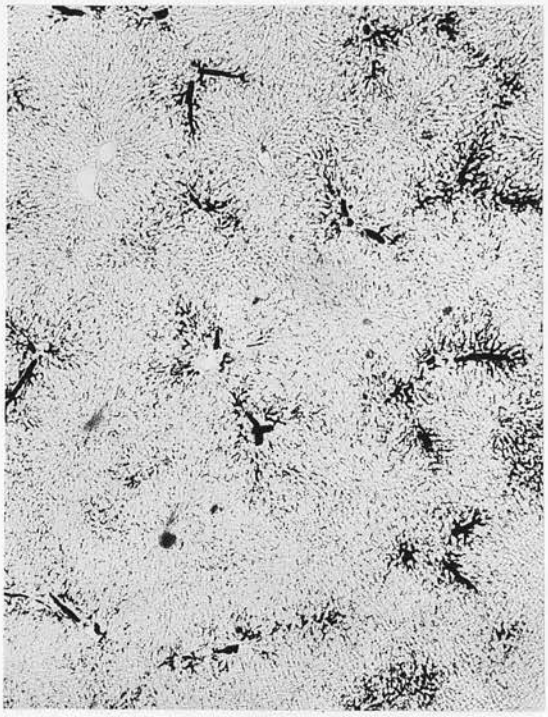


Fig.129. Rat liver, 50 micron section. Photoarteriograph, X30. 4 hr mercury specimen. The picture resembles that present at 1 hr(fig.128).Note the obstructive dilatation of the portal vessels.

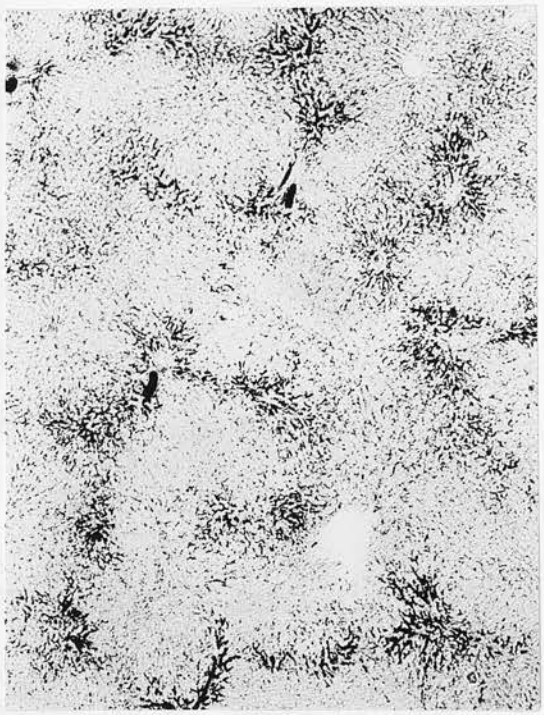


Fig.130. Rat liver, 50 micron section. Photoarteriograph, X30. 6 hrs after mercury. Minimal penetration of bismuth particles is noted. Compare with figs. 128 and 129.

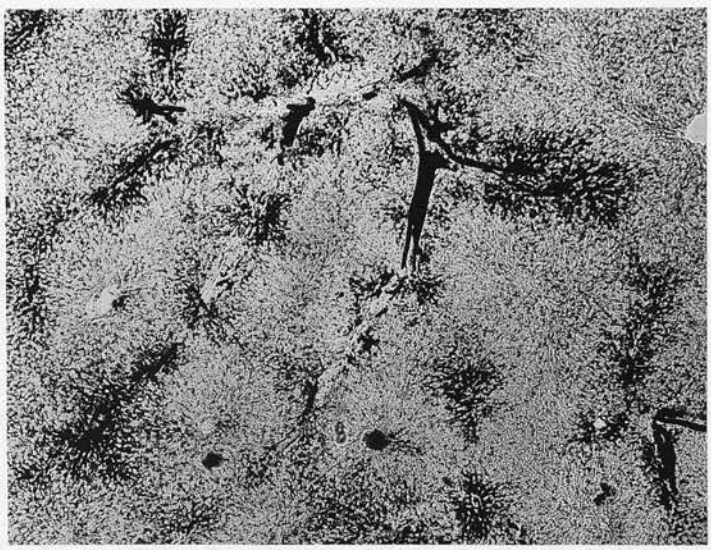


Fig.131. Rat liver, 50 micron section. Photoarteriograph, X30. 9 hrs after $HgCl_2$. Same animal as shown in fig.125. Note that there is still a considerable degree of sinu-:soidal ischemia, though there has been some return of intralobular blood-flow. Obstructive changes are still visible in the pre-sinusoidal vessels.

The hepatic vascular changes in mercury poisoning are thus seen to be very similar to the alteration imposed by carbon tetrachloride. There is an immediate obstruction to the intralobular blood flow which lasts for at least 9 hours, with some suggestion that the ischemia may be decreasing slightly by the end of this period.

Summary of the findings in Experiment 2.

1. Renal changes following the administration of 0.5 mgs of mercuric chloride appear to be those of peri-:tubular capillary ischemia, as outlined with bismuth oxychloride contrast medium. The results are disappoint-:ing, due to unavoidable and unforseen agglomeration of the bismuth medium employed. The ischemia is seen between 4 and 9 hours in the series studied, being absent in the specimen viewed 1 hour after mercury intoxication.

2. The above changes are visualized best in the 250 micron sections enlarged 20 times. The 50 micron sections do not contain sufficient bismuth medium to allow of accurate intercomparison.

3. X-rays of 700 micron sections and of whole kidneys with bismuth contrast at 9 hours following mercuric chloride suggest a widespread cortical ischemia in the kidney.

4. The use of 20% vermillion as a contrast medium results in excellent filling of the peritubular capillaries in the 4 control animals, as seen by means of X30 enlarge-:ments of 50 micron sections. The kidneys of the two 4-hour mercurialized animals show a moderate ischemia of the cortical capillary network in comparison.

5. The renal studies, based on 17 animals injected with bismuth and 6 animals injected with vermillion, cannot be interpreted as more than suggestive of peri-:tubular capillary ischemia. Intensive future studies are indicated.

6. The hepatic studies are based on the observations from 34 animals. The changes here are very similar to those seen in acute carbon tetrachloride intoxication. There is an acute ischemia of the intralobular circu-:lation plus an obstructive distension of the pre-sinusoidal arteriolar leashes in portal areas in all mercurialized animals as compared to all control animals.

7. No differences are observed between experimental and control groups in luminal diameters of the larger hepatic blood vessels from whole liver arteriographs enlarged twice.

8. As shown in Section IV, Experiment 2, the impedence to intralobular flow is a direct result of swelling of the hepatic parenchymal cells.

9. Hepatic ischemia is present throughout the intervals studied (1 to 9 hours), but there is a suggestion that it is starting to alleviate by the latter interval.

The study which follows is related only indirectly to the general plan of the investigation. The opportunity arose to undertake an arteriographic study on the kidney of a human case of acute renal failure. The case is incorporated here mainly from the point of view of general interest.

Experiment 3; arteriographic and histologic studies of a human autopsy case of acute and subacute tubular necrosis with focal infarction following upon mitral valvulotomy and acute hypotension.

Clinical Summary. Mr. P.W., Aet.23., Sex, Male.

This 23 year old man was admitted to the Eastern General Hospital under the care of Mr. Logan's thoracic

unit on the 9th of November, 1953. He had a known mitral-insufficiency of 2 year's duration, with some calcification of the mitral valve as observed under the x-ray screen. The heart was not appreciably enlarged, nor was there any pulse irregularity, but for 2 years he had complained of increasing dyspnea on exertion which had progressed to orthopnea within the past 6 months.

A mitral valvuloplasty was performed on November 11th, 1953, during the course of which the blood loss was moderate (44 ounces) and was largely replaced by a 36 ounce transfusion during the operation. His immediate post-operative condition seemed fair, with regular pulse and a systolic blood-pressure of 80 mms Hg. The chest was noted to be draining blood slowly but continuously. During the next 4 hours a total external blood loss of 5 pints was recorded, and the patient was returned to the theatre where the wound was re-opened. The blood-pressure at this time was 65/40. Overnight, a further 2 pints of blood were lost and replaced. By morning he had regained consciousness, with the pulse-rate at 130/min., regular, and the blood-pressure at 95/80. He was noted to have developed a partial right-hemiplegia. During the early post-operative period he was transfused with

a total of 9 pints of whole blood. On the 2nd post-operative day (12 Nov.) he became severely oliguric, passing 5 ounces of heavily blood-stained urine. By the following day (13 Nov.) the urine was less bloody and totalled 4 ounces. A well-marked jaundice had appeared, and the patient was sweating profusely, drowsy and incoherent. It was suspected that an incompatible transfusion had possibly been given, but there was no proof, hematologically or biochemically that such had occurred. The patient was placed on Bull's regime of gastric drip (glucose, peanut-oil and acacia in 1½ litres of water daily). His condition remained unchanged until his death on the 19th November, 1953, 9 days following the operation, apart from the disappearance of the jaundice by the 16th of November.

Laboratory findings. Urinalyses revealed constant, heavy albuminuria (up to 2.2 gms / 100 ccs) throughout the interval. In the specimen obtained on November 13th, there were several R.B.C's in the urinary sediment after centrifugation, but the supernatant urine was free from hemoglobin and hemoglobin derivatives by spectroscopic examination. The specific gravity remained high and fixed (range, 1.017 to 1.021).

Blood Chemistry.	Nov.13.	Nov.14.	Nov.17.	Nov.19.
Plasma Chloride (as NaCl).	89m.Eq./l.	91m.Eq./l.	86m.Eq./l.	
Blood Urea Nitrogen.	99 mgs%.	123 mgs%.	188 mgs%.	287 mgs%.
Plasma CO_2 - C-P.	59 vols%.		33 vols%.	30 vols %.
Serum Calcium.	9.6 mgs%.			
Plasma Phosphorus.	5.4 mgs%.			
Serum Bilirubin.	3.8 mgs%.	1.4 mgs%.		
Serum Sodium.	124m.Eq./l.	133m.Eq./l.	117m.Eq./l.	133m.Eq/l.
Serum Potassium.	6.0m.Eq./l.	6.1m.Eq./l.	5.7m.Eq./l.	7.5m.Eq./l.
Blood Sugar.				178 mgs%.

Autopsy record. M.H.A. 5455. The body was reported to be free of gross external changes such as edema. The site of the thoracotomy wound was apparent. There were approximately 10 ounces of blood in each pleural cavity. The pericardium showed the operative site. The heart was removed intact and supplied to the surgeons, along with the pulmonary vessels and lungs. Abnormalities in the gastro-intestinal tract were limited to the liver, which was stated to be deeply congested, with ill-defined hepatic lobules. In the

urinary tract, there was no abnormality of the pelves or ureters. The bladder was thick-walled and showed congestion of the mucosa in the region of the trigone. The kidneys were reported as follows: "Both kidneys were of normal shape and average size. They were firm. The capsules were translucent except at the upper pole of the left kidney where the capsule was stained a deep reddish black colour where the underlying kidney had been stained ? as a result of an embolic infarct. The capsule stripped easily from this kidney and on section it showed a well-defined cortex and medulla with no reduction in the depth of cortex. There was no thickening of the vessels. (The other kidney was sent out intact for arteriographic studies)".

Microscopical examination revealed the changes of chronic passive congestion (thickened alveolar septa, hemosiderin-laden histiocytes, etc) in the lungs; rheumatic pancarditis (Aschoff bodies, fibrosis, etc); cloudy swelling and ischemia of the hepatic lobules, with early degenerative changes in the centres of the lobules. The renal changes were said to be those of a "lower nephron nephrosis." End of Autopsy Protocol MHA. 5455.

Procedure. The 360 gram, pale, edematous-looking right kidney was received approximately 4 hours after death, with the renal artery intact and attached to the aorta. A cannula was inserted into the anterior and posterior segments separately, and the kidney was flushed with approximately 300 ccs of heparinized saline, followed immediately by bismuth contrast medium until flow had ceased. The renal arteries and vein were occluded with a large string ligature, and the kidney was x-rayed in the fresh state. It was fixed in formalin for 24 hours and then hemi-sectioned. A block of tissue was removed into Helly's fluid for histological purposes and the two halves were then post-fixed in formalin for a week. A small, recent infarct was present at one pole on one of the halves. This half was embedded in gelatin and frozen solid in a deep-freeze. It was then sectioned at 700 microns on a special large-section microtome. The section was subsequently x-rayed and prints of 5-times enlargements were prepared.

Results. A. Arteriographic. The full thickness kidney x-ray, natural size, is illustrated in fig. 133.

A control kidney from a case of acute myocardial infarction with death on the third day is shown in fig. 132. This patient had been on phenylindane-:dione and heparin before death. Autopsy 24 hours after death. The contrast medium in the control is 20% vermillion in methyl cellulose suspension; that of the tubular necrosis is the standard bismuth suspension used elsewhere in this investigation.

Fig.132. Radioarteriograph of control human kidney, natural size. Mr. W.G., male, aet 69. Acute myocardial infarction 3 days before death. On heparin and phenylin-:danedione. Kidney weight, 200 gms. Kidney injected 24 hours after death with 20% vermillion medium.

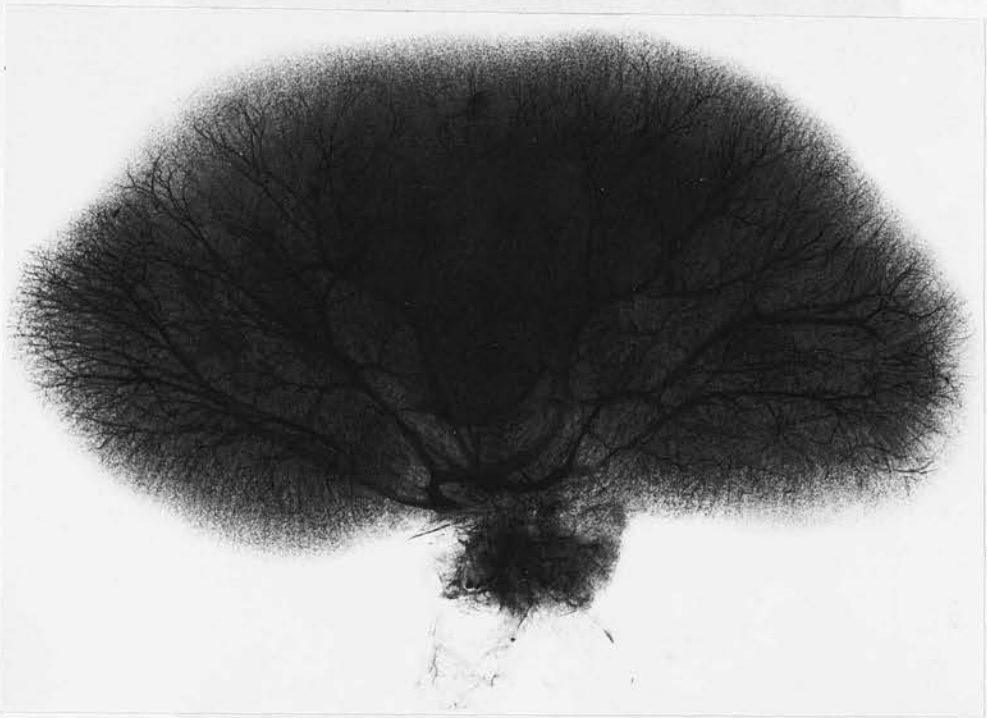


Fig.133. Radioarteriograph of kidney from the case of acute tubular necrosis detailed in the text. Note the narrow arterial lumina and large over-all dimensions. Natural size, weight 360 gms. Injected with bismuth contrast medium 4 hours after death.

There is appreciable narrowing of the entire arterial tree in the diseased kidney, due to the extensive edema in the organ. The entire kidney is obviously much enlarged. Differences in contrast and definition between the two kidneys is due, in large part, to variations in the moisture content and thickness of the structures. A radioarteriograph of the 700 micron section through the infarct at one pole is shown in fig. 135, enlarged approximately 3 times. The infarcted zone is sharply demarcated as a pale, wedge-

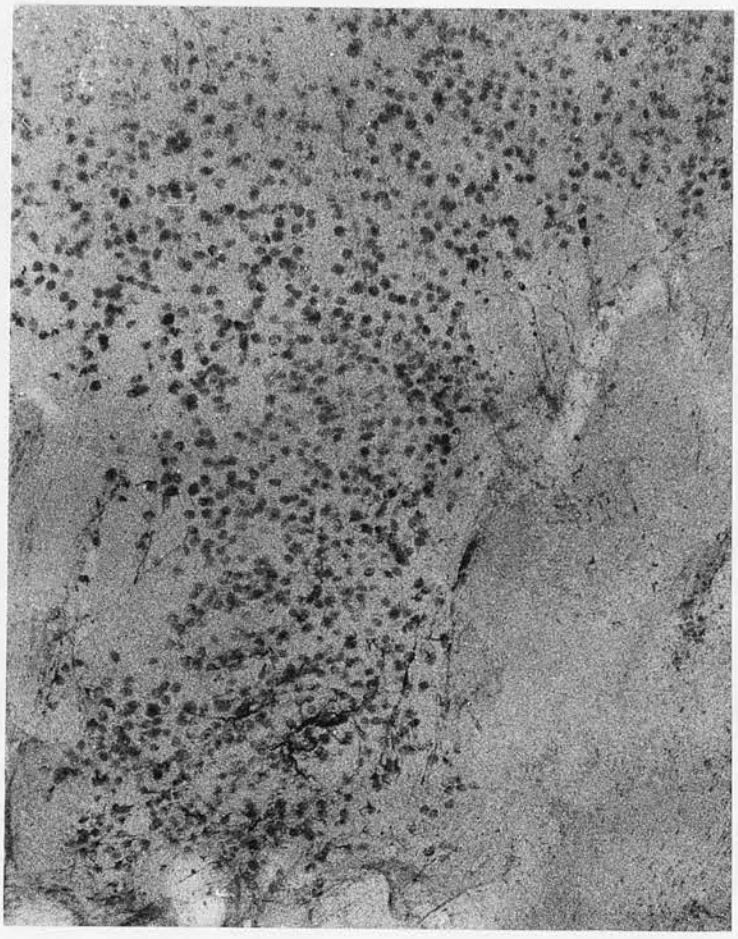


Fig.134. Radioarteriograph of 700 micron section X5. Control kidney, same case as fig. 132. There is better filling of cortical glomeruli than seen in fig. 135.

shaped area containing one poorly-injected intra-:lobular artery and a few glomeruli. Superficial cortical glomeruli are present elsewhere in a patchy distribution. It must be emphasized that the kidney was not injected until 4 hours had elapsed from the time of death. The patient had received no anti-coagulant, and there could be no guarantee of adequate filling of the vascular tree. The 700 micron sectional x-ray of the control kidney is shown in fig. 134.

Fig. 135. Radioarteriograph of 700 micron section, X3. Kidney from the case of "lower nephron nephrosis" illustrated in fig. 133. Note the well delineated infarct at the pole, also the patchy contrast-filling of the superficial cortex.

B. Histological. The renal features are typical of acute and subacute tubular necrosis. Acute necrosis is found to a moderate extent in proximal and distal tubular epithelia. Protein granular, and cellular casts are widespread in distribution, but are most numerous in the distal convoluted tubules. The infarcted zone shows the typical wedge-shaped zone of total necrosis marginated by leukocytes and vascular hyperemia. The section through this particular area was taken after a week's

formalin-fixation and following the x-ray studies. It is poorly fixed and does not lend itself to detailed microscopic study. In the remainder of the cortex, the sections are well preserved. Extensive evidence of former tubulorrhexis is strikingly dis-played in the proximal convoluted tubules, where irregular piling-up of regenerating epithelium practically obliterates the lumina of many tubules. The surrounding interstitium is edematous. There is marked hyaline-droplet degeneration, and this change is even present in the epithelial cells of the glomeru-lar tuft and of Bowman's capsule. Most of these changes are well shown in fig. 136. The hepatic lesion consists of a widespread centrolobular necrosis, with small round cell infiltration of the necrotic zones (fig. 137). This case is an excellent example of acute tubular necrosis (or G.T.N.) which was precipitated by acute hypotension consequent upon practical exsanguination. The embolic episodes (cerebral and renal) are doubtless due to the primary disease (rheumatic heart disease) with atrial thrombus formation and embolization. Certain theoretical aspects are detailed in the general discussion which follows.

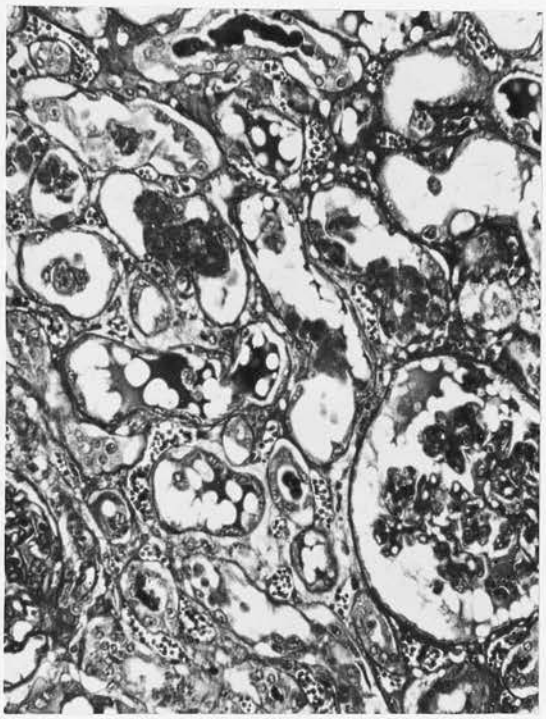


Fig. 136. Human kidney. Tri.X200. Case of "lower nephron nephro-:sis" (acute and subacute tubular necrosis). Note evidence of tubulorrhexis in the proximal tubules. Also the marked hyaline-droplet degeneration of epi-:thelial cells of the glomerulus and tubules plus extensive cast formation.

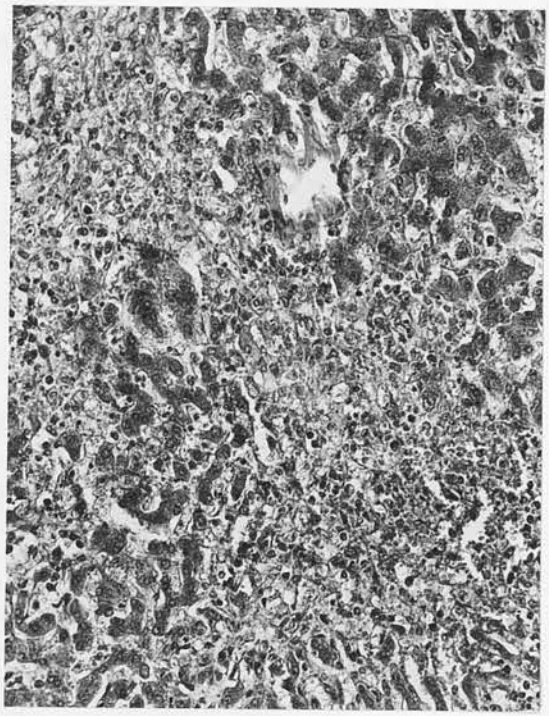


Fig.137. Human liver. H&E X150. Same case as in fig. 135. There is a very extensive degree of centro-:lobular necrosis, with moderate small round cell infiltration.

Discussion.

As emphasized throughout this section, the changes observed in the intrarenal circulation cannot be accepted without the proverbial "grain of salt". Deeming it wise to err on the side of under- rather than over-valuation, I am assessing the renal changes observed with carbon tetrachloride as having an

80% chance of being significant, and similarly, the renal changes in mercury poisoning as having a 65% chance. The findings in the liver under both poisons are well established and reliable. Obviously, the kidney changes require a great deal of further investigation before any definite conclusions may be drawn, such as; 1. the choice of a new medium with a particle size of good uniformity which will be small enough to enter the normal peritubular capillary bed and large enough to be excluded from an ischemic capillary bed; 2. the unequivocal proof of the ischemic nature of these lesions and the actual site of the vasospasm; and 3. the actual time of onset and release of ischemia in CCl_4, $HgCl_2$ and pituitrin nephroses, assuming that ischemia does, in fact, occur.

From the arteriographic investigations of the rat kidney, it seems not unlikely that spasm occurs between 1 and 4 hours after mercurial intoxication and at some time prior to 4 hours in carbon tetrachloride poisoning. Such spasm as is suggested by these results would appear to operate at the level of the efferent arteriole of the glomeruli in the earliest stages, since at these intervals the glomeruli and afferent vessels are well filled with contrast medium, with a slight increase in the glomerular diameters

suggesting incomplete obstruction beyond the level of the glomerular tuft. That this could occur without spasm is entirely feasible, providing there be a marked degree of swelling of cells lining the peritubular capillaries. Such is certainly not the case in regard to the vascular endothelium, and I can make no histological case for swelling of the tubular epithelium prior to 6 hours (see Section IV, Experiments 1 and 2). Indeed, even at this interval, such cloudy swelling as does occur is intraluminal and does not appear to occlude the peritubular blood channels. As discussed in Section IV, Allen (1951) considers the efferent arteriole of the cortical glomerulus to be the most likely site for any spastic process to seize upon, and this is in agreement with the observation referred to above, that the site must lie between the glomerulus and the ischemic capillary bed. On the other hand, the localization of 50% of the mercury lesions to the cortico-medullary zone at lower dosages (Section IV) suggests that the cortical efferent arterioles are certainly no more prone to spasm than are the cortico-medullary efferent arterioles. This throws some suspicion upon the accuracy of the micro-anatomy of the renal blood supply

as proposed in fig. 6,c, (cf. Trueta et al, 1947), particularly in regard to that of the rat. As indicated in the introduction to this work, no hard and fast rule in regard to the relative diameters of the two types of efferent arterioles could be elicited by direct observation from the present studies in the rat.

Renal ischemia, in CCl_4 damage, never progresses beyond the patchy peritubular ischemia illustrated in Experiment 1, Section V. The mercury alteration, on the other hand, shows a decidedly greater filling defect after 9 hours, the ischemic process extending back to involve glomeruli, afferent arterioles and even intralobular arteries. This could be effected in one of two ways. Either the larger vessels themselves go into spasm, or the spastic change at the efferent arteriole is of such severity and duration that total obstruction to the flow of blood occurs, the process spreading in extent to efface the supply area of the intralobular artery affected. No evidence of arterial thrombosis is noted histologically, but an acute fibrinoid arteritis does occur (Section IV, Experiment 2). A not dissimilar arteritis is seen in malignant hypertension, as noted by Allen (1951) in connection with human mercurial nephrosis.

Sheehan and Moore (1952) consider the vasospasm of renal cortical necrosis to be initiated at the glomerular level and to spread proximally to involve the larger vessels, depending upon the degree and duration of the spasm (unpublished data mentioned in their above monograph). If this be proven, then it likewise appeals as the most likely pathogenetic explanation of the observed phenomena in mercurial nephrosis.

With regard to the arteriographic observations of hepatic changes in CCl_4 and mercurial intoxication, we stand on more secure ground. With both poisons the change is similar, namely, acute swelling of the parenchymal cells forming the walls of the vascular sinusoids. As a result of this swelling there is an acute ischemia of the intralobular circulation which is present at the earliest interval studied in this series, i.e. at 1 hour. Comparison of the livers illustrated in Experiments 1 and 2 would suggest that the hepatic ischemia in mercury poisoning is more extreme, though of possibly shorter duration, than its CCl_4 counterpart. On the other hand, a few differences do exist, and these deserve mention. Firstly, the swelling from carbon tetrachloride, as shown in Section IV, Experiment 1, is limited in my

series to the inner $\frac{2}{3}$s of the liver lobules; that due to mercuric chloride involves every cell in the hepatic cords. Secondly, the vacuolar degenerative change of the parenchymal cell, though not dissimilar in its lobular distribution, appears more severe in CCl_4 injury. Thirdly, centrolobular necrosis, while a feature of CCl_4 poisoning, does not occur in livers with mercury poisoning in this series. (It is an infrequent finding in the same condition in human livers (cf. Ogilvie, 1932)). Nonetheless it is highly probable that the acute mercury lesion is more incapacitating to liver function than is the acute CCl_4 lesion. Every liver cell is affected, and presumably is influenced both by blockage of its glutathione (respiratory) enzyme systems and by acute, ischemic hypoxia. The normal appearing cells of the (large) outer $\frac{1}{3}$ of the lobule in CCl_4 intoxication doubtless help to ameliorate the functional derange-:ment of the liver in this condition. The absence of changes in the lumen of the portal vein in acute CCl_4 intoxication is confirmed by the identical 'in-vivo' findings of Daniel et al, 1952.

As indicated in the introductory remarks to this section, the method of microarteriography employed in these investigations is new and hitherto untried.

Until Bellman's (1953) monograph became available in February of 1954, no comparable technique was procurable for intercomparison. It is thus gratifying to learn that he also has used a ganglion-blocking agent (hexamethonium) with success. On the other hand, from the results of vasospastic angiographic experiments in the ears of living rabbits, he has shown that occasionally wetting agents are capable of themselves producing arterial spasm. I have used Ilford Wetting Agent as a basic ingredient of both the bismuth and the vermillion contrast media employed in this investigation. It may possibly account for the absence of significant changes in the lumina of the major renal and hepatic vessels between the experimental and control groups. It does not influence the findings of sinusoidal and capillary obstruction in Experiments 1 and 2, Section V, since these changes are largely confined to the poisoned rats and absent in the control animals. Bellman originally employed a freshly prepared solution of 20% silver iodide (Parke-Davis, Neoprotosil) as recommended by Barclay (1951), but more recently he has been using 35% diodrast (3,5-di-iodo, 4-pyrido-N-acetic diethanolamine) almost exclusively, finding it reasonably well tolerated by the living animal. Such iodinated

hydrocarbons have obviously no utility in the technique which I have developed, for they are in molecular solution and are diffusible. They present no opacity to the wavelengths of light.

Experiment 3, Section V, is included primarily as an interesting human case in which both arteriographic and histologic studies have been made. The findings fully support the ischemic nature of infarction and suggest a mild remnant of ischemia in a subacute nephro-:sis related etiologically to a prior acute hypotensive state. The case also presents a definite hepato-renal correlation, though the significance of the hepatic changes is difficult to assess. In any long-standing cardiac failure, the liver is inevitably rendered hypoxic and develops atrophic or necrotic lesions. In this instance there was superimposed the acute anoxic state associated with excessive hemorrhage, exaggerating the liver damage and producing acute renal failure (and see Moon, 1948). The liver may or may not have played some pathogenetic role in the renal lesions which developed, and I do not see how one could prove this point at the present state of our knowledge. Perhaps it would be reasonable to suggest that of the two primary etiological agents of acute renal failure co-existing in this individual, the renal lesion was

precipitated by hypotensive anoxia and aggravated and protracted by the hepatic necrosis and insufficiency incurred during the same hypotensive episode in a liver possibly already precariously near the borderline of hepatic decompensation due to chronic hypoxia. The above line of reasoning is purely speculative, but should it prove correct, it has implications in the understand-:ing, prevention and therapy of acute renal failure, regardless of the precipitating cause, e.g. shock, toxemia of pregnancy, chemical poisons, etc.

During the discussion of the findings which evolved from the investigations undertaken in Section IV, (experiments which had been planned from prior obser-:vations on human autopsy material and early experi-:mental studies into acute hepatic ischemia in rabbits (Sections II and III)), the following hypothesis was proposed:

1. The liver is a key in a homeostatic, humoral system for the regulation of the circulation, denaturing vaso-active materials such as pituitrin which are in constant production and are ever present in the systemic circulation.

2. The damaged liver fails to denature such materials, resulting in a rise in their titre in the circu-:lating blood.

3. Such vasospastic substances produce vascular spasm throughout the body, their effect being most pronounced in certain organs which, by reason of some idiosyncrasy in their vascular anatomy are

rendered markedly ischemic by spasm. The commonest of such sites are the kidneys and the utero-placental junction.

This hypothesis was formerly founded on the widely accepted but theoretical assumption that acute tubular necrosis is of ischemic, vasospastic etiology. The results of the present series of microarteriographic studies suggest that ischemia is the cause of the tubular necrosis which accompanies the carbon tetra-:chloride and mercurial forms of nephrosis. Though actual spasm is not demonstrated, the evidence favours a spastic form of occlusion, with the efferent arteriole as the site of obstruction. Further weight is lent to the hepato-renal hypothesis by the convincing arteriographic demonstration of early hepatic ischemia at the level of the intralobular circulation, both in mercuric chloride and carbon tetrachloride intoxi-:cation. The hypothesis is illustrated diagrammatically in fig. 138. It will be appreciated that hepatic damage may well introduce a vicious cycle which will tend to propagate the hepatic lesion via spastic ischemia. This suggestion is postulated from the well-known fact that repeated massive doses of pituitrin do cause hepatic necrosis (cf. Fauvet, 1931; Vegh and von Pallos, 1937; Govan and Mukherjee, 1950, b.). It is, of course, improbable that any such concentration of the hormone would be found in the blood stream under the

proposed conditions, but the idea merits consideration.

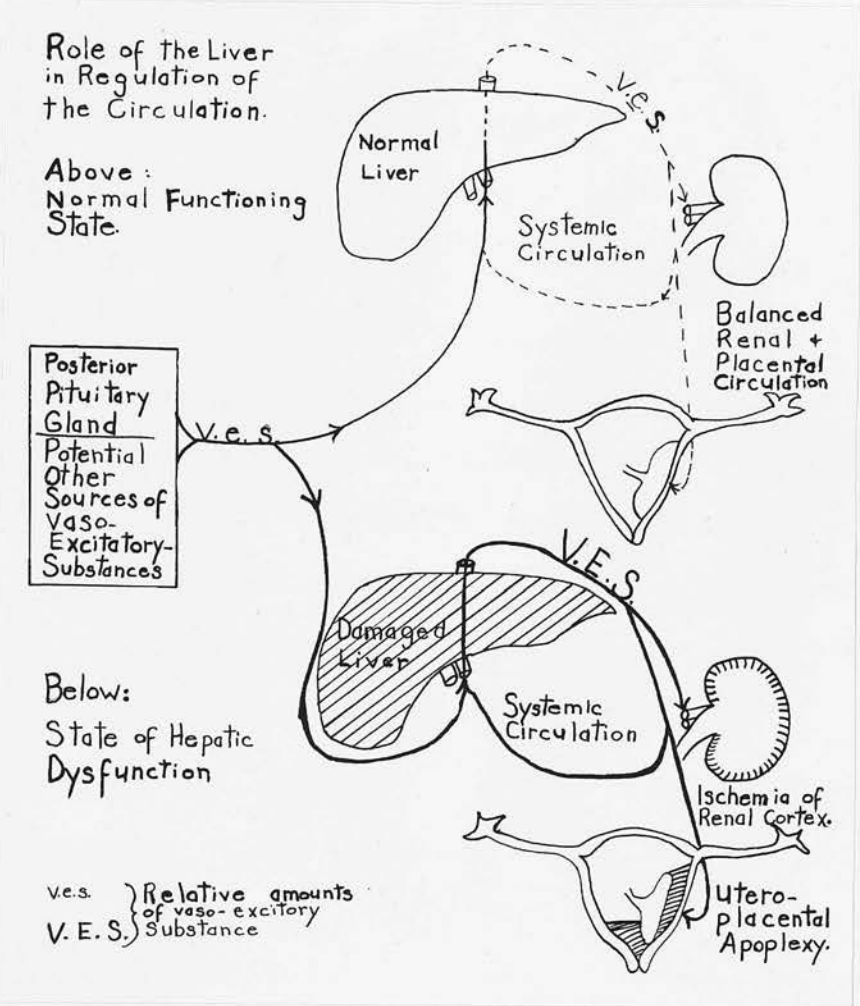


Fig. 138. The proposed role of the liver in the humoral regulation of the circulation. Above, the state of normal homeostasis. Below, the mechanics of the hepato-:renal syndrome.

This hypothesis is possibly much over-simplified, (cf. Shorr et al, 1945 and 1947; and Delorme, 1951). The latter found that oxygenation of the portal blood protected dogs against severe, exsanguinative, hypotensive states. He postulates that the oxygenated liver produces a pressor substance or its precursor (hypertensinogen); participates in subsidiary pressor mechanisms; and actively destroys vasodepressor substances. The anoxic liver, he suggests, fails to produce protective pressor compounds; actively forms vasodilator material; and fails to destroy circulating depressor material.

Such arguments, of course, are based exclusively on findings in conditions of shock where an acute hypotensive episode occurs and the kidneys and liver are inevitably ischemic. The suggestion that hepatic anoxia results in a failure of the liver to produce vaso-excitatory substances and an active production, with failure to denature of a vaso-depressor material, appears incompatible with the findings of this thesis. Here, hepatic anoxia has been shown to potentiate the vaso-excitatory principle of posterior-pituitary extract, resulting in a vast increase in its necrotizing activity towards the renal tubules. In addition,

the morphologically identical carbon tetrachloride lesion has here been shown to most likely result from a vasospastic change at the level of the renal efferent arteriole. It is in no way explicable on the basis of vasodilatation at this or any other level. Block et al, (1952, a), attribute the renal tubular necrosis, seen in their dogs with prolonged experimental hypo-:tension, to vasospasm plus decreased blood-pressure and blood-flow in the kidneys. They state that the lesion is independent of renal innervation and that the Trueta shunt mechanism is not demonstrable. (It should be added that no evidence of a cortico-medullary arterio-venous shunt was seen in my entire series of investigations in the rat). If, as Block suggests, the renal lesion in shock is of spastic etiology and independent of renal innervation, the pathological changes in the liver and kidneys fit into my proposed scheme remarkably well. On the other hand, the patho-:genesis of hemorrhagic shock is very incompletely understood. For instance, as admitted by Shorr et al, 1945, the production of a vasodilator material by liver and skeletal muscle in severe, hemorrhagic, hypotensive states occurred only in their anesthetized dogs. In the unanesthetized condition, the animals never became hypo-reactive (to adrenalin) and never

produced a circulating vasodilator material. The anesthetic they employed was sodium pentobarbital, an agent normally detoxified by the liver. On the other hand, they have demonstrated the production of vaso-:dilator and vasopressor materials from tissue slices under anaerobic conditions and they present evidence suggesting that the adrenal cortex plays some role in the findings. These agents, V.E.M. and V.D.M., were assayed with regard to their ability to respectively potentiate or inhibit the activity of adrenalin on the metarterioles of normal rat meso-appendix. In other words, the vascular activity of these compounds was mediated indirectly through the vasopressor effect of adrenalin, acting in the capacity of an accelerator or a brake. Thus the humoral regulatory system for the control of the circulation proposed by these authors would appear to act, if at all, purely to modify the neurigenic (sympathetic) response to the shocking agent. The experimental approach to the present investigations was formulated with the avoidance of shock as a major consideration. Thus, none of my results bear directly on the shock state.

The schema shown in fig. 138 must be considered only a framework for future amplification. It is built upon personally observed facts of correlated

hepatic damage and renal G.T.N. in humans, rabbits and rats in a wide variety of clinical states; of the probable ischemic nature of the renal lesion designated as G.T.N.; and of the marked increase in the severity of G.T.N. produced by pituitrin in rats with liver damage. It also incorporates observations of numerous other workers in the field of eclamptic toxemia of pregnancy. It is incomplete in regard to forms of vaso-excitatory substances other than pituitrin; to possible humoral vaso-depressor substances inactivated by the normal liver; to a possible interplay of metabolic liver functions (protein, fat, carbohydrate and bile); to the question of renal vaso-active substances, both in normal and ischemic kidneys; and to a large number of sites of untoward vasospastic activity. However, this hypothesis is capable of very broad application and appears most adequately to explain the facts which have been presented throughout this text. It is, indeed, a highly expanded concept of the "hepato-renal syndrome".

Summary.

1. A microarteriographic procedure, using heavy-metal blood-replacement media during the agonal phase, is described. Its limitations are discussed.

2. Arteriospasm in the larger renal vessels is absent in pituitrin, carbon tetrachloride and mercuric chloride nephroses in the rat.

3. Technical difficulties arose in radiographic procedures which will require much of the work to be repeated for confirmation.

4. Despite their potential unreliability, the results suggest that vasospasm occurs at the level of the efferent arteriole in the early stages of carbon tetrachloride and mercury nephrosis.

5. Such vasospasm produces ischemia of the peritubular capillaries sometime between 1 and 4 hours after giving mercury and at some time prior to 4 hours following CCl_4 inhalation.

6. Between 4 and 24 hours, the CCl_4 spasm and renal ischemia is consistently patchy in nature. It disappears by 30 hours.

7. The mercurial vasospasm is similar to the above in the 4 and 6 hour renal change, but becomes more severe and extensive by 9 hours, obliterating many glomeruli, afferent arterioles and interlobular arteries.

8. Evidence for some obstruction in the hepatic intralobular circulation was found after 1 hour in both CCl_4 and $HgCl_2$ poisoning. The obstruction results from swelling of the hepatic parenchymal cells.

9. Intralobular ischemia persists in slowly decreasing severity for 36 hours in CCl_4 intoxication. The mercuric chloride ischemia shows some suggestion of a decrease in 2 out of 6 animals after 9 hours (the last interval studied).

10. As a consequence of this restriction of intralobular blood-flow, the pre-sinusoidal arteriolar leashes in the portal areas become distended and give an excellent anatomical display.

11. The effect of mercury on the liver is considered to be more severe than that of carbon tetrachloride, but the duration of the ischemic state is less.

12. The autopsy findings in a patient dying from acute tubular necrosis are discussed, the case having well-correlated hepatic and renal lesions, suggesting a possible double cause in the production of the renal damage.

13. It is suggested that the liver is a key organ in a humoral regulatory system for the maintenance of the circulation. This suggestion is also presented in a simple diagrammatic form.

14. This hypothesis may help to explain the many entities which have, as clinical and pathological features, acute renal failure and some variety of acute tubular necrosis of ischemic origin, plus some morpho-:logical derangement of the liver.

15. The postulate constitutes a broadened conception of the so-called "hepato-renal syndrome".

BIBLIOGRAPHY

Bibliography.

1. Allen, Arthur C.: The Kidney: Medical and Surgical Diseases. Grune and Stratton. New York. 1951.

2. Andrews, W.H.H.: The liver lesions in malaria. Trans. of Soc. Trop. Med.; 41: 699, 1948.

3. Barclay, A.E.: Microarteriography. Blackwell Scientific Publications. Oxford. 1951.

4. Baxter, J.H. and Ashworth, C.T.: Renal lesions in portal cirrhosis. Arch. Path.; 41: 476, 1946.

5. Beall, D., Bywaters, E.G.L., Belsey, R.H. and Miles, J.A.R.: A case of crush injury with renal failure. Brit. M.J.; 1: 432, 1941.

6. Bellman, S.: Microangiography. Acta Radiol. Supplementum 102. Stockholm, 1953.

7. Block, M.A., Wakim, K.G., Mann, F.C. and Bennett, W.A.: Renal lesions and function following prolonged experimental hypotension. Surgery; 32: 551, 1952. (a).

8. Block, M.A., Wakim, K.G. and Mann, F.C.: Effect of severe acute hemorrhage on kidney of rat. Arch. Path.; 54: 443, 1952 (b).

9. Bohatyrtschuk, F.: Uber ergebnisse der mikroroentgenographie. Acta Radiol. 25: 351, 1944.

10. Boyce, F.F. and McFetridge, E.M.: So-called "liver-death". Arch. Surg.; 31: 105, 1935.

11. Bradley, H.C.: Autolysis and atrophy. Physiol. Rev.; 18: 173, 1938.

12. Bull, J.M., Joekes, A.M. and Lowe, K.G.: Renal function studies in acute tubular necrosis. Clin.Sci.; 9: 379, 1950.

13. Bull, G.M. and Dible, J.H.: Section on renal diseases in Hadfield, G.: Recent advances in pathology, 6th ed., J. & A. Churchill Ltd. London. 1953.

14. Bywaters, E.G.L. and Beall, D.: Crush injuries with impairment of renal function. Brit. M.J.; 1: 427, 1941.

15. Bywaters, E.G.L.: Crushing injury. Brit. M.J.; 2: 643, 1942.

16. Bywaters, E.G.L. and Dible, J.H.: The renal lesion in traumatic anuria. J. Path. and Bact.; 54: 111, 1942.

17. Bywaters, E.G.L. and Dible, J.H.: Acute paralytic myohemoglobinuria in man. J. Path. and Bact.; 55: 7, 1943.

18. Cameron, G.R. and Karunaratne, W.A.E.: Carbon tetrachloride cirrhosis in relation to liver regeneration. J. Path. and Bact.; 42: 1, 1936.

19. Cameron, G.R.: Normal and pathological patterns in the liver. In "Studies in pathology", presented to Peter MacCallum. Melbourne University Press. Carlton, N.3, Victoria. 1950.

20. Campbell, A.C.P. and Henderson, J.L.: Symmetrical cortical necrosis of the kidneys in infancy and childhood. Arch. of Dis. in Child.; 24: 269, 1949.

21. Chandler, A.C. and Chopra, R.N.: The toxicity of CCl_4 to cats: A warning. Indian M. Gaz.; 60: 406, 1925.

22. Cruikshank, J.: The histological appearances occurring in organs undergoing autolysis. J. Path. and Bact.; 16: 167, 1912.

23. Daniel, P.M., Prichard, M.M.L. and Reynell, P.C.: The portal circulation in experimental cirrhosis of the liver. J. Path. and Bact.; 64: 53, 1952.

24. Delorme, E.J.: Arterial perfusion of the liver in shock. An experimental study. Lancet. 1: 259, 1951.

25. Diekmann, W.J. and Michel, H.L.: Vascular-renal effects of posterior pituitary extracts in pregnant women. Am. J. Obst. Gynec.; 33: 131, 1937.

26. Duff, G.L. and More, R.H.: Bilateral cortical necrosis of the kidneys. Am. J. of Med. Sci.; 201: 428, 1941.

27. Edwards, J.G.: The renal tubule (nephron) as affected by mercury. Am. J. Path.; 18: 1011, 1942.

28. Epstein, F.H., Lesser, G.T. and Berger, E.Y.: Renal function in decompensated cirrhosis of the liver. Proc. Soc. Exper. Biol. and Med.; 75: 822, 1950.

29. Eschenbrenner, A.B. and Miller, E.: Sex differences in kidney morphology & chloroform necrosis. Science; 102: 302, 1945.

30. Fahr, T.: Cholämische nephrose. In Henke, F. and Lubarsch, O.: Handbuch der speziellen pathologischen anatomie und histologie. Julius Springer, Berlin. 6: Teil 1, 281, 1925.

31. Farquhar, J.D.: Renal studies in acute infectious hepatitis. Am. J. Med. Sci.; 218: 291, 1949.

32. Fauvet, E.: Hypophysenhinterlappenhormone und schwangerschaftstoxikosen. Klin. Wschr. 10: 2125, 1931.

33. French, A.J.: Glomerulonephrosis, morphologic manifestation of renal cortical ischemia in toxic oliguria and lower nephron nephrosis. Arch. Path.; 49: 43, 1950.

34. Furtwaengler, A.: Diffuse rindennecrose beider nieren nach leberruptur. Krankheitsforschung; 4: 349, 1927.

35. Gardner, G.H., Grove, R.C., Gustafson, R.K., Mauri, E.D., Thompson, M.J., Wells, H.S. and Lamson, P.D.: Studies on the pathological histology of experimental CCl_4 poisoning. Bull. Johns Hopkins Hosp.; 36: 107,1925.

36. Glynn, L.E. and Himsworth, H.P.: The intralobular circulation in acute liver injury by carbon tetra-:chloride. Clin. Sci.; 6: 235, 1948.

37. Goby, P.: Une application nouvelle des rayons x: la microradiographie. Compt. rend. Acad. d. sc.; 156; 686, 1913.

38. Govan, A.D.T. and Mukherjee, C.L.: The vascular supply to the liver and the anatomy of eclampsia. Brit. J. Exp. Path.; 31: 485, 1950 (a).

39. Govan, A.D.T. and Mukherjee, C.L.: The synergistic action of the anterior and posterior pituitary hormones and their possible relation to eclampsia. Brit. J. Exp. Path.; 31: 626, 1950 (b).

40. Grechishkin, S.V. and Prives, M.G.: Weiche roentgen-:strahlen in medizin und embryologie. Vestnik rentgen. i. radiol; 14: 201, 1935. (Quoted by Bellman, 1953).

41. György, P., Seifter, J., Tomarelli, R.M. and Gold-:blatt, H.: Influence of dietary factors and sex on the toxicity of CCl_4 in rats. J. Exp. Med.; 83:449,1946.

42. Hall, M.C. and Shillinger, J.E.: Miscellaneous tests of CCl_4 as an anthelmintic. J. Agric. Res.; 23: 163, 1923.

43. Harmon, E.L.: Human mercuric chloride poisoning by intravenous injection. Am. J. Path.; 4: 321, 1928.

44. Heintzelmann, F.: Hepatitis- renal function tests in patients with reference to the hepato-renal syndrome. Nord. med.; 33: 240, 1947. (Quoted in Snavely, J.R.: Hepatic factors in salt and water metabolism. Am. J. Med. Sci.; 223: 96, 1952).

45. Helwig, F. and Schutz, C.: A liver-kidney syndrome. Surg., Gyn. and Obs.; 55: 570, 1932.

46. Herdman, J.P. and Jaco, N.T.: The effect of renal artery constriction on the renal blood flow. Brit. J. Exp. Path.; 31: 806, 1950.

47. Himsworth, H.P.: The liver and its diseases. 2nd ed. Blackwell Scientific Publications. Oxford. 1950.

48. Jaffé, R.H. and Sternberg, H.: Uber die vakuoläre nieren-degeneration bei chronischer ruhr. Virchow's Arch.; 227: 313, 1919.

49. Jennings, R.B. and Kearns, W.M. Jr.: Necrotizing nephrosis in the rat following administration of CCl_4. Arch. Path.; 56: 348, 1953.

50. Jensen, E.J., Baggenstoss, A.H. and Bargen, J.A.: Renal lesions associated with ulcerative colitis. Am. J. Med. Sci.; 219: 281, 1950.

51. Kulka, J.P., Pearson, C.M. and Robbins, S.L.: A distinctive vacuolar nephropathy associated with intestinal disease. Am. J. Path.; 26: 349, 1950.

52. Lamarque, P.: Historadiographie. Compt. rend. Acad. d. sc.; 202: 684, 1936.

53. Loeffler, L. and Nordmann, M.: Leberstudien, Teil 1. Virchow. Arch. 257: 119, 1925.

54. Lucké, B.: Lower nephron nephrosis. The military surgeon; 99: 371, 1946.

55. Mauro, G.: Intossicazione de tetracoruro di carbonio. Clin. med. ital. 61: 192, 1930. (Quoted in Jennings and Kearns, 1953).

56. Mayneord, W.V.: The radiography of the human body with radioactive isotopes. Brit. J. Rad.; 25: 517, 1952.

57. McManus, J.F.A.: The periodic acid routine applied to the kidney. Am. J. Path.; 24: 643, 1948.

58. Mönninghof, F.H.: Untersuchungen uber die autolyse der zellen bei "trüber schwellung" und "post-mortaler kadaveröser trübung". Beit. zur. Path. Anat.; Ziegler. 102: 87, 1939.

59. Moon, V.H.: The pathology of secondary shock. Am. J. Path.; 24: 235, 1948.

60. Moon, H.D.: The pathology of fatal CCl_4 poisoning, with special reference to the histogenesis of the hepatic and renal changes. Am. J. Path.; 26: 1041, 1950.

61. More, R.H. and Duff, G.L.: The renal arterial vasculature in man. Am. J.Path.; 27: 95, 1951.

62. Morrione, T.G. and Mamelock. H.L.: Observations on the persistence of hepatic glycogen after death. Am. J. Path.; 28: 497, 1952.

63. Morrison, L.M.: Improvement in kidney function following therapy for cirrhosis of the liver. Rev. Gastroenterol.; 14: 533, 1947.

64. Norcross, J.W., Feldman, J.D., Bradley, R.F. Jr. and White, R.M.: Liver function: an attempt to correlate structural change with functional abnor-:mality. Ann. Int. Med.; 35: 1110, 1951.

65. Ogilvie, R.F.: The pathological changes produced in the tissues by corrosive sublimate with special reference to the early phases of cell degeneration and to changes in the blood fat. J. Path. and Bact.; 35: 743, 1932.

66. Ogilvie, R.F.: The alkali reserve and fat content of the blood. Edin. M. J. New Series (IVth); 41: 448, 1934.

67. Oka.: Zur frage der postmortalen autolyse der zellgranula. Virchow Arch.; 228: 200, 1920.

68. Oliver, J., MacDowell, M. and Tracy, A.: The patho-:genesis of acute renal failure. J. Clin. Investigation.; 30(2): 1307, 1951.

69. Opie, E.L.: The effect of injury by toxic agents upon osmotic pressure maintained by cells of liver and kidney. J. Exper. Med.; 91: 285, 1950.

70. Patek, A.J. Jr., Seegal, D. and Bevans, M.: The coexistence of cirrhosis of the liver and glomerulo-:nephritis. Am. J. Med. Sci.; 221: 77, 1951.

71. Pytel, A.: Zur frage des hepato-renalen syndromes. (Experimentelle untersuchung). Arch. Klin. Chir.; 187: 27, 1936.

72. Rhoads, C.P., Van Slyke, D.O., Hiller, A. and Alving, A.S.: Effects of novocainization and total section of nerves of renal pedicle on renal blood flow and function. Am. J. Physiol.; 110: 392, 1934.

73. Scriver, W. DeM. and Oertel, H.J.: Necrotic sequestration of the kidneys in pregnancy (symmetrical cortical necrosis); clinical and anatomico-pathogenetic study. J. Path. and Bact.; 33: 1071, 1930.

74. Seneviratne, R.D.: Physiological and pathological responses in the blood-vessels of the liver. Quart. J. Exp. Phys.; 35: 77, 1949.

75. Sheehan, H.L. and Moore, H.C.: Renal cortical necrosis and the kidney of concealed accidental hemorrhage. Blackwell Scientific Publications. Oxford. 1952.

76. Shorr, E., Zweifach, B.W. and Furchgott, R.F.: On the occurrence, sites and modes of origin and destruction of principles affecting the compensatory vascular mechanisms in experimental shock. Science; 102: 489, 1945.

77. Shorr, E., Zweifach, B.W., Furchgott, R.F. and Baez, S.: Hepatorenal factors in experimental hypertension. In 'Factors regulating blood pressure'. Transactions of the 1st conference, April 24-25, 1947. New York. Josiah Macy Jr. Foundation.

78. Smillie, W.G. and Pessoa, S.B.: Treatment of hookworm disease with CCl_4. Am. J. Hyg.; 3: 35, 1923.

79. Smyth, H.F., Smyth, H.F. Jr. and Carpenter, C.P.: The chronic toxicity of CCl_4: Animal exposures and field studies. J. Indust. Hyg. and Toxicol.; 18: 277, 1936.

80. Tomb, J.W.: Anuria and anoxia. J. Trop. Med. Hyg.; 45: 125, 1942.

81. Toreson, W.E.: Glycogen infiltration (so-called hydropic degeneration) in the pancreas in human and experimental diabetes mellitus. Am. J. Path.; 27:327, 1951.

82. Trowell, O.A.: The experimental production of watery vacuolation of the liver. J. Physiol.; 105: 268, 1946.

83. Trueta, J., Barclay, A.E., Daniel, P.M., Franklin, K.J. and Prichard, M.M.L.: Studies of the renal circulation. Blackwell Scientific Publications. Oxford. 1947.

84. van Beek, C. and Haex, A.J. Ch.: The part of post-mortal autolysis in the necrosis of the liver parenchyma in subacute atrophy. Acta Med. Scand.; 114: 557, 1943.

85. Végh, L. and von Pallos, K.: Histologische verande-:rungen bei tieren nach behandlung mit grossen mengen von hypophysen-hinterlappenextrakten. Klin. Wchncsh.; 16: 1536, 1937.

86. Wainright, J.: Tubular necrosis following temporary occlusion of the renal artery in the rat. Brit. J. Exp. Path.; 31: 400, 1950.

87. Wakim, K.G. and Mann, F.C.: Effect of experimental cirrhosis on the intrahepatic circulation of blood in the intact animal. Arch. Path.; 33: 198, 1942.

88. Waters, L.L. and Stock, C.B.: B A L. (British Anti-Lewisite). Science; 102: 601, 1945.

APPENDIX

TABLE 1

TABLE I

50 Cases of glomerulotubular nephrosis & hepatic damage from the K.G.H. in the years 1948 & 1951-52.

\+ = Renal lesions - which were recorded on the autopsy protocol.

TABLE I

50 Cases of glomerulotubular nephrosis & hepatic damage from the K.G.H. in the years 1948 & 1951-52

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded Additional Renal Lesion +	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
1.	A-8-48.	Acute Encepha-litis		Fatty Metamor-phosis & C.P.C.	Nil.	320	2050	+	22	F	1½
2.	A-25-48.	Appendi-citis. Post-op. shock & Periton-itis.	Acute gan-grenous Appendi-citis.	Focal Necrosis. Fatty Metamor-phosis.	Nil.	350	1950	+	52	M	6
3.	A-45-48.	Carcinoma of Rectum	Pulmonary Edema. Broncho-pneumonia.	Centro-lobular atrophy & Necrosis.	Moderate arterio-sclerosis.	425	1600	+	69	M	2

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt. of Kidneys in GMS.	Wt. of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
4.	A-47-48.	Burns. Shock.	Acute Broncho-pneumonia.	Centro-lobular Necrosis.	Lower Nephron Nephrosis.	450	2150	+	47	M	?
5.	A-50-48.	Subdia-phragma-tic Abscess.	Acute Diffuse Periton-itis.	Acute Edema & Focal Necrosis.	Lower Nephron Nephrosis.	300	2100	-	55	M	9
6.	A-61-48.	Carcinoma of large Intestine.	Secondary Carcinoma of Brain.	Extreme C.P.C.	Nil	300	1550	-	45	F	6½
7.	A-65-48.	Hyper-tensive & Coronary Heart Disease.	Acute Broncho-pneumonia.	Centro-lobular Necrosis & marked C.P.C.	Nil.	365	1625	-	70	M	8
8.	A-67-48.	Carcinoma of Pancreas.	Biliary Cirrhosis	Biliary Cirrhosis	Bile Nephrosis of Kidney.	275	1300	-	74	F	1

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded Additional Renal Lesion	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
9.	A-71-48.	Massive Pulmonary Embolism.	Generalised Arterio-sclerosis.	Severe C.P.C.	Nil.	180	1125	-	92	M	3½
10.	A-79-48.	Hyper-tensive Heart Disease.	Myocardial Infarction.	Severe C.P.C.	Nil.	275	1350	-	50	M	?
11.	A-82-48.	Carcinoma of Large Intestine.	Acute Diffuse Peritonitis.	Severe C.P.C.& Centro-lobular Necrosis.	Nil.	255	1650	-	69	M	?
12.	A-88-48.	Coronary Heart Disease.	Arterio-sclerosis of Coronary Arteries.	Severe C.P.C.	Nil.	230	1500	-	64	M	2

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
13.	A-92-48.	Acute Pancrea-titis.	Periton-itis.	Cirrhosis, Fatty Metamor-phosis & C.P.C.	Nil.	335	2150	-	26	M	$2\frac{1}{2}$
14.	A-93-48	Fracture of Skull. Edema of Brain.	Portal Cirrhosis.	Cirrhosis, Fatty Metamor-phosis & Focal Necrosis.	Nil.	355	2325	+	33	M	4
15.	A-105-48.	Carcinoma of Thyroid.	Secondary Ca. of Lungs, Lymph Node, Superior Vena Cava.	Moderate C.P.C.	Nil.	120	1080	-	66	M	?
16.	A-107-48.	Addison's Disease.	T.B. of Adrenals (Bilateral).	Focal Necrosis & Edema.	Misdiag-nosed as Cortical Necrosis	225	1225	+	54	F	8

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded+ Additional Renal Lesion	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
17.	A-118-48.	Rheumatic Heart Disease.	Severe Mitral Stenosis.	Severe C.P.C.& Centro-lobular Necrosis.	Nil.	410	1800	-	48	M	½
18.	A-120-48.	Chronic Myelogenous Leukaemia.	Thrombosis of Pulmon-ary Artery.	Leukaemia, Infiltrat-ion & Mod-erate C.P.C.	Nil.	300	2500	-	27	M	¾
19.	A-121-48.	Cholodocho-lithiasis. Biliary Cirrhosis.	Ruptured Esophageal Varices (terminal).	Biliary Cirrhosis & Focal Necrosis.	Nil.	375	1700	-	47	F	6½
20.	A-122-48.	Typhoid Fever.		Focal Necrosis. Cholangio-hepatitis.	Nil.	438	2160	-	15	M	5

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
21.	A-126-48.	Adenocarcinoma of Colon.	Subdiaphragmatic Abscess.	Centrolobular Necrosis & Atrophy.	Nil.	325	2000	+	68	M	10
22.	A-132-48.	Portal Cirrhosis.	Ruptured Esophageal Varices (terminal).	Cirrhosis & Focal Necrosis. Acute Hepatitis.	Nil.	250	1950	-	41	F	6½
23.	A-135-48.	Congenital Malformation of Heart.	Acute Myocardial Failure.	Severe C.P.C. & Centrolobular Necrosis.	Nil.	275	1250	-	36	F	1
24.	A-146-48.	Idiopathic Hypertrophy of Heart.	Myocardial Failure.	Severe C.P.C. & Fatty Metamorphosis.	Nil.	625	250	-	6 mos.	F	3

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt. of Kidneys in GMS.	Wt. of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
25	A-159-48.	Carcinoma of Gall Bladder.	Biliary Cirrhosis (early).	Tumor & Cirrhosis.	Bile Nephrosis.	250	2850	-	77	F	10
26.	A-162-48.	Duodenal Ulcer.	Portal Cirrhosis of Liver.	Cirrhosis & Focal Necrosis.	Nil.	425	2200	+	53	F	1¾
27.	A-164-48.	Congenital Heart Disease.		Centro-lobular Necrosis.	Cortical Necrosis (minimal).	20	855	-	2 dys.	F	2
28.	A-346.	Reticulum Cell Sarcoma.	Anemia (Hgb-5.6gms.)	C.P.C.	Nil.	250	1350	?	72	M	1
29.	A-353.	Alcoholism. Portal Cirrhosis.	Hemorrhaging Peptic Ulcer.	Cirrhosis & Focal Necrosis.	Nil.	450	1700	-	60	M	12

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
30.	A-373.	Peptic Ulcer. Gastrectomy.	Massive Atelectasis.	Centrolobular Necrosis.	Nil.	350	2200	-	49	M	8
31.	A-394.	Pernicious Anaemia.	Acute Yellow Atrophy of Liver.	Massive Hepatic Necrosis & Bile Retention.	Bile Nephrosis.	300	850	-	52	F	½
32.	A-402.	Carcinoma of Stomach.	Carcinoma of Prostate.	Tumor & Fatty Metamorphosis.	Arteriosclerotic scarring (slight)	325	1700	+	87	M	5
33.	A-411.	Subdural Hematoma.	Alcoholism.	Cirrhosis & Fatty Metamorphosis.	Arteriosclerotic scarring (slight)	350	2100	-	66	M	13

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
34.	A-421.	Perforation of Ileum.	Peritonitis.	Cloudy Swelling.	Arterio-sclerotic scarring (slight)	202	1200	-	73	M	12
35.	A-446.	Thymoma.	Cirrhosis of Liver.	Cirrhosis & C.P.C.	Nil.	250	1000	-	47	M	6
36.	A-449.	Acute Broncho-pneumonia.	Acute Nephrosis & Cerebral Thrombosis.	Acute Edema & C.P.C.	Regenerating Nephrosis.	30	100	?	5 wks	M	11
37.	A-468.	Peptic Ulcer. Gastrectomy.	Bile Peritonitis.	Focal Necrosis. Acute Edema & Fatty Metamorphosis.	Lower Nephron Nephrosis.	400	1800	-	49	M	½

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
38.	M.L.M.	Alcoholism.	Portal Cirrhosis.	Cirrhosis.	Arterio-sclerotic scarring (slight)	-	-	-	65	M	12
39.	M.L.P.	Alcoholism.	Acute Nephrosis.	Edema & Fatty Metamor-phosis.	Acute Nephrosis.	440	2550	-	30	M	10
40.	A-455.	Lutem-bacher's Disease.	Cardiac Cirrhosis.	Cirrhosis & Centro-lobular Necrosis.	C.P.C.	300	1400	-	44	F	1
41.	A-405.	Embolism of Femoral Artery.	Involut-ional Melan-cholia.	Centro-lobular Necrosis.	Arterio-sclerotic scarring (slight)	170	750	-	67	F	3

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded+ Additional Renal Lesion	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
42.	A-477.	Coronary Heart Disease.	Diabetes.	Centro-lobular Necrosis & Atrophy.	Nil.	440	1800	-	57	F	8
43.	A-480.	Pulmonary Fibrosis. Healed T.B.	Cor Pulmonale.	Centro-lobular Atrophy, Necrosis & Fibrosis.	Nil.	400	1250	-	47	M	4
44.	A-487.	Coronary Thrombosis.	Myocardial Failure.	Edema & C.P.C.	Nil.	400	2000	-	65	M	1½
45.	A-493.	Pulmonary T.B.	Cachexia.	Early Cirrhosis, Focal Necrosis & C.P.C.	Nil.	250	1050	-	31	F	10
46.	A-497.	Carcinoma of Breast.	Hepatic Coma.	Atrophy & Marked Secondary Tumor	"Bile Nephrosis"	350	4700	-	52	F	12

Table 1 contd.

Case	Number	Primary Disease	Associated Diseases	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt.of Kidneys in GMS.	Wt.of Liver in GMS.	Shock	Age	Sex	Hrs. P.M.
47.	A-499.	Hepatic Centro-lobular Necrosis.	Toxic (?)	Centro-lobular Necrosis - con-fluent.	? Toxic Nephrosis	100	350	-	13 mos	M	6
48.	A-504.	Pulmonary Fibrosis & Cor Pul-monale.	Cardiac Cirrhosis.	Centro-lobular Fibrosis, Necrosis, Atrophy & Congest-ion.	Nil.	350	1175	-	48	M	4
49.	A-505.	Carcinoma of the head of the Pancreas, Roux-Y.	Gangrene of Small Bowel.	Centro-lobular Necrosis. Bile Stasis.	Slight Arterio-sclerosis.	320	1200	+?	67	M	7
50.	A-506.	Trauma. Skull Fracture.	Intra-cerebral Haemorrhage.	Centro-lobular Necrosis.	Lower Nephron Nephrosis.	395	2050	-	19	M	10

TABLE 2

TABLE 2

Control Series (15 cases) from Kingston, Toronto & Edinburgh since 1948.

+ = Renal lesions - which were recorded on the autopsy protocol.

TABLE 2.

Control Series (15 cases) from Kingston, Toronto & Edinburgh since 1948.

Case	Number	Primary Disease	Associated Disease	Hepatic Lesion	Recorded Additional Renal Lesion	Wt. of Kidneys	Wt. of Liver	Shock	Age	Sex	Hrs. P.M.	Refrig-eration.
1.	K.G.H. A90-49	Traumatic Aortic Rupture.	--	Slight Terminal Congest-ion.	Nil.	-	-	+	15	M	4	+
2.	K.G.H. A234.	Pulmonary Tubercu-losis.	Thoracen-tesis and Sudden Death.	Slight Centro-lobular Atrophy.	--	-	-	-	36	M	5	+
3.	K.G.H. A303.	Cerebral Haemorr-hage.	--	Slight Fatty Metamor-phosis.	Moderate arterio-sclerosis.	-	-	-	72	F	10	+
4.	H.D.H. -2.A433.	Congenital Heart Disease.	--	Nil.	Medullary Hyperemia.	-	-	-	S.B.	F.	12	-

XV

Table 2 contd.

Case	Number	Primary Disease	Associated Disease	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt.of Kidneys	Wt.of Liver	Shock	Age	Sex	Hrs. P.M.	Refrig-eration.
5.	Toronto M.L.6.	Fractured Skull.	Extra-dural haemorr-hage.	Slight fatty Metamor-phosis.	Nil.	-	-	?	37	M	10	+
6.	R.H.S.C. 48 - 16.	Trauma-tic Death.	--	Nil.	Nil.	Avge.	Avge.	?	4	F	24	-
7.	R.H.S.C. 49 - 97.	Bacillary Dysen-tery.	(Sonne)	Slight Terminal Congest-ion.	Nil.	Avge.	Avge.	-	4	F	4	-
8.	R.H.S.C. 51 - 78.	Virus Pneumonia.	--	Slight Terminal Congest-ion.	Nil.	Avge.	Avge.	-	$2\frac{1}{2}$	M	8	-
9.	R.H.S.C. 52 - 3.	Severe Scalds.	Acute Heart Failure.	Terminal Acute Congest-ion.	Nil.	Avge.	Avge.	+	3	F	24	-

Table 2 contd.

Case	Number	Primary Disease	Associated Disease	Hepatic Lesion	Recorded + Additional Renal Lesion	Wt.of Kidneys	Wt.of Liver	Shock	Age	Sex	Hrs. P.M.	Refrig-eration.
10.	R.H.S.C. 52 - 46.	Virus Pneumonia.	--	Slight Terminal Congestion.	Nil.	Avge.	Avge.	-	2	F	12	-
11.	L.H.A. 582.	Traumatic Death Within 2 hrs.	--	Moderate Autolysis.	Moderate Autolysis.	Avge.	Avge.	?	41	M	48	+
12.	E.H.A. 1391.	Post-op erative Periton-eal Ad-hesions.	Intestinal Obstruction.	Moderate-ly Ad-vanced Autolysis.	Moderate-ly Ad-vanced Autolysis.	Avge.	Avge.	?	48	F	72	-
13.	E.H.A. 1465.	Acute Rheumatic Fever.	Acute Cardiac Failure.	Terminal Acute Congest-ion.	Acute Congest-ion.	Avge.	Sl.en-larged	-	$3\frac{1}{2}$	M	24	-

Table 2 contd.

Case	Number	Primary Disease	Associated Disease	Hepatic Lesion	Recorded[+] Additional Renal Lesion	Wt.of Kidneys	Wt.of Liver	Shock	Age	Sex	Hrs. P.M.	Refrig-eration.
14.	E.H.A. 1694.	Traumatic Death.	Rupture of Spleen and Lungs. Intra-cranial Haemorrhage.	Nil.	Nil.	Avge.	Avge.	+	17	M	24	-
15.	M.H.A. 4274.	Femoral Phlebo-thrombosis.	Massive Pulmonary Embolus.	Terminal Acute Congest-ion.	Terminal Congest-ion.	Avge.	Avge.	-	34	F	5	-

TABLE 3

TABLE 3

The Lesions of Glomerulo-tubular Nephrosis.

An analysis of 50 cases from the K.G.H. autopsy files in the years 1948 and 1951-52, and of 15 control cases from Kingston, Toronto, and Edinburgh.

+ Non-specific Nephrosis (see text).

TABLE 3.

THE LESIONS OF GLOMERULO-TUBULAR NEPHROSIS.

An analysis of 50 cases from the K.G.H. autopsy files in the years 1948 and 1951-52, and of 15 control cases from Kingston, Toronto and Edinburgh.

Renal Lesions	Lower Nephron Nephrosis, 5 cases.		Bile, Toxic, etc. Nephrosis, 8 cases.		N-S Nephrosis 37 cases.		Total		Controls	
	No.	%.	No.	%.	No.	%.	No.	%.	No.	%
1. Glomerulus										
(a) exudate into capsular space	4.	80%	7.	88%	36.	97%	47.	94%	8.	54%
(b) swelling of capsular epithelium	5.	100%	8.	100%	34.	92%	47.	94%	3.	20%
(c) increased cellularity of tuft..	0.	0%	2.	25%	3.	8%	5.	10%	0.	0%
(d) epithelial desquamation	5.	100%	8.	100%	37.	100%	50.	100%	6.	40%
(e) epithelial crescents	0.	0%	0.	0%	0.	0%	0.	0%	0.	0%
2. Proximal tubules										
(a) dilatation of lumina	4.	80%	6.	75%	31.	84%	41.	82%	1.	7%
(b) pyknosis of nuclei	5.	100%	5.	63.5%	37.	100%	47.	94%	3.	20%
(c) Coagulation of cytoplasm	5.	100%	7.	88%	36.	97%	48.	96%	1.	7%
(d) cellular and amorphous casts ..	5.	100%	7.	88%	37.	100%	49.	98%	1.	7%
(e) epithelial desquamation	3.	60%	5.	63%	35.	95%	43.	86%	1.	7%
3. Thin limb of loop of Henle										
(a) hyaline casts	5.	100%	8.	100%	37.	100%	50.	100%	4.	27%

Table 3 contd.

Renal Lesions	Lower Nephron Nephrosis, 5 cases.		Bile, Toxic, etc. Nephrosis, 8 cases.		N-S Nephrosis + 37 cases.		Total		Controls	
	No.	%.	No.	%.	No.	%.	No.	%.	No.	%
4. Distal tubules (including thick limb of Henle)										
(a) pyknosis of nuclei	5.	100%	8.	100%	34.	92%	47.	94%	8.	54%
(b) coagulation of cytoplasm ...	4.	80%	7.	88%	29.	78%	40.	80%	3.	20%
(c) dilatation of lumina	5.	100%	8.	100%	34.	92%	47.	94%	4.	27%
(d) cellular casts	5.	100%	8.	100%	35.	95%	48.	96%	1.	7%
(e) hyaline casts	5.	100%	7.	88%	37.	100%	49.	98%	4.	27%
(f) granular casts	5.	100%	8.	100%	27.	77%	40.	80%	4.	27%
(g) pigmented casts										
i. bile	0.	0%	4.	50%	0.	0%	4.	8%	0.	0%
ii. hyalo- &/or granular-heme	5.	100%	1.	13%	20.	54%	26.	52%	2.	13%
(h) epithelial desquamation	4.	80%	5.	63%	26.	70%	35.	70%	8.	54%
(i) fatty vacuolization	1.	20%	4.	50%	13.	35%	18.	36%	0.	0%
(j) tubal rupture	3.	60%	1.	13%	0.	0%	4.	8%	0.	0%
(k) regeneration	2.	40%	1.	13%	0.	0%	3.	6%	0.	0%
5. Collecting tubules										
(a) Casts	5.	100%	7.	87.5%	36.	97%	48.	96%	1.	7%

Table 3 contd.

Renal Lesions	Lower Nephron Nephrosis, 5 cases.		Bile, Toxic, etc. Nephrosis, 8 cases.		N-S Nephrosis+ 37 cases.		Total		Controls	
	No.	%.	No.	%.	No.	%.	No.	%	No.	%
6. Interstitium										
(a) edema	5.	100%	6.	75%	25.	68%	36.	72%	1.	7%
(b) inflammatory cell infiltration	4.	80%	4.	50%	8.	22%	16.	32%	0.	0%
(c) granulomatous reaction	4.	80%	2.	25%	1.	3%	7.	14%	0.	0%
7. Blood vessels										
(a) angiitis	3.	60%	1.	13%	1.	3%	5.	10%	0.	0%
(b) cortical collapse and medullary hyperemia	2.	40%	1.	13%	0.	0%	3.	6%	0.	0%

TABLE 4

TABLE 4

The Incidence of Experimental and Natural G.T.N.[x]
in Rabbits.

+ = Killed and Autopsied.
x = Glomerulotubular nephrosis.

Degree:-	
Absent	-
Minimal Acute GTN	+
Moderate Acute GTN	++
Moderate Subacute GTN	+++
Advanced Subacute GTN	++++

Table 4.

The Incidence of Experimental and Natural G.T.N.[x] in Rabbits.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt. in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urin-alysis.	Degree of x G.T.N.
A	A22	Nil	Coccidiodosis	1860	K & A[+]	Coccidiodal mycosis & focal necrosis	-	+
	A23	Nil	Coccidiodosis	2030	K & A	Coccidiodal mycosis & focal necrosis & portal fibrosis	-	+++
	B13	Nil	Coccidiodosis	2300	K & A	Coccidiodal mycosis(extreme)	-	+
	B15	Nil	? Infectious Hepatitis	1320	K & A	Serous hepatitis & centrolobular necrosis	-	+
	B16	Nil	? Infectious Hepatitis	1350	K & A	Centrolobular necrosis	-	++

Medical Diseases of Liver (8)

Table 4 contd.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt. in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urin-alysis.	Degree of x G.T.N.
Medical Diseases of Liver (8) A	B19	Nil	Coccidiodosis	2200	K & A	Coccidiodal mycosis(slight)	-	-
	B23	Nil	? Infectious Hepatitis	2300	K & A	Focal necrosis (marked)	-	++
	B24	Nil	Coccidiodosis	2170	K & A	Coccidiodal mycosis(extreme)	-	++++
Medical & Surgical Diseases of Liver (13) B	98	Porta clamped 30 mins.	? Sub-acute Hepatitis	1920	Operative Death	Periportal fibrosis(early)	-	+
	A1	Porta clamped 23 mins.	? Infectious Hepatitis	2010	Operative Death	Focal necrosis (patchy)	-	+++
	A4	Portal vein Ligated.	Coccidiodosis	2020	Early Post-op. Death	Focal necrosis (massive) & Coccidiodosis	-	+++

Table 4 contd.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt. in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urin-alysis.	Degree of x G.T.N.
B	A6	Hepatic artery Ligated.	Coccidiodosis	2050	Death 8 hrs Post-op.	Centrolobular necrosis & Coccidiodal mycosis	-	+
Medical & Surgical Diseases of Liver (13)	A27	Laparotomy for 20 mins.	? Acute and Sub-acute Hepatitis.	1950	K & A - 24 hrs Post-op.	Focal necrosis (patchy) and Periportal fibrosis	Neg.	+
	A28	Laparotomy for 20 mins.	Coccidiodosis	2100	Operative Death	Focal necrosis (patchy) and Coccidiodal mycosis	-	++
	A38	Porta clam-ped 15 mins.	Coccidiodosis	1730	Operative Death	Focal necrosis (patchy) and Coccidiodal mycosis	-	+

Table 4 contd.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt.in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urin-alysis.	Degree of x G.T.N.
B	A39	Porta clamped 18 mins.	Coccidiodosis	1350	K & A - 24 hrs Post-op.	Focal necrosis (massive) and Coccidiodal mycosis	Alb.+ Bile.tr. R.B.C)2 HPF Casts)0.1	+
Medical & Surgical Diseases of Liver (13)	A41	Porta clamped 14 mins.	? Acute & Subacute Hepatitis.	1980	Operative Death	Focal necrosis (patchy) and Periportal fibrosis	-	++++
	A42	Porta clamped 20 mins.	? Infectious Hepatitis.	1860	Operative Death	Centrolobular necrosis	-	+
	A43	Porta clamped 20 mins.	? Infectious Hepatitis.	1730	Operative Death	Centrolobular necrosis	-	+
	A35	Porta clamped 20 mins.	Coccidiodosis	2480	K & A - 24 hrs Post-op.	Focal necrosis (patchy) and Coccidiodal mycosis	Alb.+ Bile.- R.B.C)0.2 HPF Casts)0.1	++

Table 4 contd.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt.in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urinalysis.	Degree of G.T.N. x
Medical & Surgical Diseases of Liver (13) B	A36	Porta clamped 15 mins.	Coccidiodosis	1760	K & A - 24 hrs Post-op.	Focal necrosis (patchy) and Coccidiodal mycosis	Alb.+ Bile - R.B.C) 0.1HPF Casts)	+
Surgical Diseases of the Liver (10) C	99	Porta clamped 30 mins.	Nil	1790	K & A - 48 hrs Post-op.	Focal necrosis (patchy)	Alb.tr Bile + R.B.C) - Casts) -	++
	A3	Portal vein Ligated.	Nil	1850	K & A - 72 hrs.	Necrosis. R. lobe - massive. M.lobe - focal.	Alb.- Bile + R.B.C) 2 HPF Casts) 0	++
	A5	Porta clamped 10 mins.	Nil	1530	K & A - 24 hrs.	Centrolobular necrosis (hemorrhagic)	Alb. + Bile + R.B.C) 0.1HPF Casts)	++

Table 4 contd.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt.in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urin-alysis.	Degree of G.T.N.[x]
C Surgical Diseases of the Liver (10)	A7	(Rt.)Hepatic Artery Ligated.	Nil	2050	K & A - 48 hrs	Necrosis R. lobe - massive. M. lobe - focal.	Alb + Bile + R.B.C)0.2 HPF Casts)0.1	++
	A8	Porta clamped 20 mins.	Nil	2150	K & A - 30 hrs Post-op.	Focal necrosis (massive)	Alb + Bile - R.B.C) 0.1 HPF Casts)	++
	A32	Porta clamped 20 mins.	Nil	1920	K & A - 24 hrs Post-op.	Focal necrosis (patchy)	Alb. tr Bile. tr R.B.C) 0.1 HPF Casts)	+
	A33	Porta clamped 20 mins.	Nil	2540	K & A - 24 hrs Post-op.	Focal necrosis (patchy)	Alb. + Bile. tr R.B.C) 0.2 HPF Casts)	++

Table 4 contd.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt.in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urin-alysis.	Degree of x G.T.N.
Surgical Diseases of the Liver (10) C	A34	Porta clamped 10 mins.	Nil	1830	K & A - 24 hrs Post-op.	Normal.	Neg.	-
	A37	Porta clamped 20 mins.	Nil	2100	K & A - 24 hrs Post-op.	Focal necrosis (patchy)	Alb. tr Bile. tr. R.B.C) 0.1 HPF Casts)	+
	A40	Porta clamped 20 mins.	Nil	2050	K & A - 24 hrs Post-op.	Focal necrosis (massive)	Alb. + Bile. tr. R.B.C) 0.1 HPF Casts) 0	+
Control Normal (9) D	A20	Nil	Nil	2050	K & A	Nil	-	-
	A21	Nil	Nil	1930	K & A	Slight chronic Periportal hepatitis	-	-
	A24	Nil	Nil	1700	K & A	Nil	-	-

XXX

Table 4 contd.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt.in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urin-alysis.	Degree of x G.T.N.
Control Normal (9) D	A25	Nil	Nil	1900	K & A	Nil	-	-
	B14	Nil	Nil	2400	K & A	Nil	-	-
	B17	Nil	Nil	2200	K & A	Nil	-	-
	B18	Nil	Nil	2500	K & A	Nil	-	-
	B20	Nil	Nil	2500	K & A	Tiny foci of ? acute degen-eration	-	+
	B21	Nil	Nil	2450	K & A	Nil	-	-

Table 4 contd.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt.in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urin-alysis.	Degree of x G.T.N.
Operative deaths and laparotomy controls (7) E	100	Porta hepatis Ligated.	Nil	1850	Operative Death	Nil	-	-
	A2	Porta clamped 6 mins.	Nil	1730	Operative Death	Nil	-	-
	A26	Laparotomy for 20 mins.	Nil	2580	K & A - 24 hrs Post-op.	Nil	Neg.	-
	A29	Laparotomy for 20 mins.	Nil	2440	K & A - 24 hrs Post-op.	Nil	Neg.	-
	A44	Laparotomy for 20 mins.	Nil	2260	K & A - 24 hrs Post-op.	Nil	Neg.	-

Table 4 contd.

Group	Rabbit Number	Surgical Procedure	Medical Diseases of Liver	Wt.in Gms.	Clinical Course	Hepatic Lesion	Post-op. Urin-alysis.	Degree of x G.T.N.
E Operative deaths and laparotomy controls (7)	A45	Laparotomy for 20 mins.	Nil	2400	K & A - 24 hrs Post-op.	Nil	Neg.	-
	A46	Laparotomy for 20 mins.	Nil	2210	K & A - 24 hrs Post-op.	Nil.	Neg.	-
F Laparotomy and occlusion of left renal artery (2)	A30	Laparotomy for 20 mins. Clamp L.renal pedicle for 10 mins.	Nil	2530	K & A - 24 hrs Post-op.	Nil	Neg.	R - L ++
	A31	Laparotomy for 20 mins. Clamp L.renal pedicle for 10 mins.	Nil	1880	K & A - 24 hrs Post-op.	Nil	Alb.tr. Bile - R.B.C.0.2 HPF	R - L ++

ABSTRACT

ABSTRACT

The finding in 1951 of three consecutive cases of renal tubular necrosis with correlated hepatic disease suggested the present series of investigations. A preliminary study was made of the changes produced by post-mortem autolysis in liver and kidney tissue. Having established the identity of pre- and post-mortem changes in the distal convoluted tubule and their dissimilarity in the proximal tubules, it was possible to formulate a specific autolytic pattern as a basis for studies in human tissues. The Masson Trichrome stain was found highly selective for degenerative epithelial changes. Unfortunately it reproduced poorly in black and white photographs.

Fifty cases with hepato-renal correlation were chosen from the autopsy files of the Kingston General Hospital over a two-year period, and from these cases was compiled the conception of "glomerulotubular nephrosis". This renal lesion was shown to consist essentially of proteinuria with acute tubular necrosis, of varying grades of severity. Of the accompanying liver lesions, roughly 50% showed necrosis, 20% cirrhosis, 20% severe fatty metamorphosis, 10% bile retention, and some changes of lesser incidence. The correlation suggested a pathogenetic link between the lesions and experimental studies were made to prove this hypothesis.

Acute, temporary hepatic ischemia was produced in rabbits by means of a clamp on the porta hepatis. Within twenty-four hours the lesion of glomerulotubular nephrosis developed with great constancy. However, a high percentage of rabbits was found to suffer from coccidiodosis of the liver, and such animals also showed the combined renal lesion. A lesion identical to glomerulotubular nephrosis was produced by direct renal ischemia. The role of shock from the operative procedure, though considered unlikely, could not be completely excluded.

The combined liver and kidney lesions caused by toxic chemicals (carbon tetrachloride and mercuric chloride) were studied in the albino rat on a serial-time basis. Both poisons were found to cause an instantaneous swelling of the liver cells with immediate hepatic ischemia. Within five hours there occurred the non-specific, ischemic, renal lesion referred to as "glomerulotubular nephrosis". A single massive dose of pituitrin was capable of producing the minimal lesion in the kidneys, but in the presence of hepatic anoxia, a sub-toxic dose of pituitrin produced uniform and very severe tubular necrosis in the kidneys.

The ischemic nature of the hepatic changes in carbon tetrachloride and mercuric chloride poisoning has been established by microarteriographic studies. In the kidneys, the results were definitely suggestive of vasospastic ischemia at the level of the efferent arteriole but this will require confirmation.

The hypothesis has been advanced that the normal liver is a key in a humoral system for the regulation of the circulation, inactivating circulating vaso-excitatory substances. With liver damage, the hepatic function of detoxification is impaired and the titre of these substances rises in the circulating blood. A generalized vasospastic change ensues, the effects being most severe in organs, which, through some peculiarity of their vascular construction may become ischemic from vasospasm. The commonest known sites of such untoward activity are the kidney and the uteroplacental junction.

++++++++++++